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Boron Binding with the Quorum Sensing Signal AI-2 and Analogues

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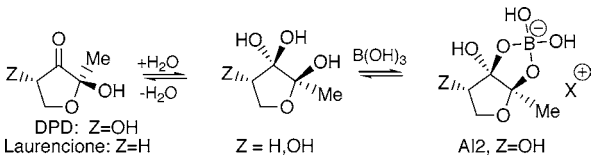
ABSTRACT



The unstable bacterial metabolic product, DPD, and the related natural product, laurencione, are shown to have a high affinity for borate complexation, through the hydrated analogue. The boron complex of DPD is *Vibrio harveyi* AI-2, an interspecies quorum sensing signal in bacteria, and an affinity column with a borate resin is effective in providing the first method for concentrating and purifying *V. harveyi* AI-2 from the biosynthetic product.

Quorum sensing is a process of cell−cell communication that populations of bacteria use to coordinate gene expression in response to fluctuations in cell density.[1] Communication is mediated by the exchange of signal molecules called autoinducers. Gram-positive bacteria use oligopeptides, and Gram-negative bacteria use acylhomoserine lactones as autoinducers for species-specific signaling.[2,3] A quorum sensing system that operates in both Gram-positive and Gram-negative bacteria uses derivatives of the metabolic product 4,5-dihydroxy-2,3-pentanedione (DPD).[4−7] Biosynthesis of DPD requires the enzyme LuxS, which is present in over 60 species of bacteria.[8,9] DPD undergoes further rearrangements to yield molecules generically termed "AI-2" (autoinducer-2) that are active in signaling.[6,7] The widespread nature of LuxS and DPD production has led to the proposal that AI-2 functions in *interspecies* communication.[1,5] AI-2 produced by many bacterial species triggers a response in the bioluminescent bacterium *V. harveyi*.[10−14] These species join a growing list of bacteria that regulate cell processes such as virulence factor production, cell motility, bacterial conjugation, and biofilm formation in an AI-2-dependent manner.[8]

The AI-2 signaling cascade has been elaborated for *V. harveyi*. From X-ray studies of the receptor protein LuxP, the structure of the signaling molecule was shown to be a hydrated furan diulose to which a cyclic borate group has been appended (**AI-2**, Figure 1).[6] Additional support for the

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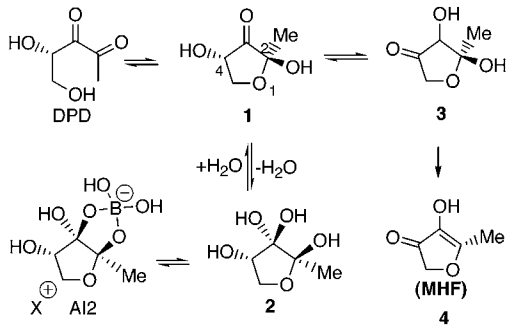

Figure 1. Reactions of DPD.

structure was a signal at δ 6.10 ppm in the ^{11}B NMR spectrum.[6] If AI-2 is released from LuxP via heat denaturation, the ^{11}B NMR signal for the released molecule shifts to δ 5.80 ppm.[7] The appearance of borate in a signaling role in bacteria is unusual and has generated considerable interest. Obvious questions concern the role of enzymes in the conversion of DPD and its cyclized form **1** to the hydrate **2** and boron complex AI-2 and whether the receptor protein LuxP is required to provide a driving force for hydration and to stabilize the borate complex. While borate binding to *cis*-cyclopentane-1,2-diols[15] and furanoses[16,17,18] is well established, we are aware of no other example where the ligand on boron derives from an α-hydroxyketone (e.g., **1**).

DPD is not a stable molecule. The cyclic form **1** is proposed to be unstable toward isomerization to **3** and then irreversible elimination to give **4** (MHF, methylhydroxyfuranone).[19] In addition, simple α-hydroxycyclopentanones have a tendency to oligomerize through acetal formation. Even though DPD has been a proposed biological intermediate for decades and qualitatively detected as a quinoxoline derivative,[18] only very recently was a synthesis published (which provides a dilute solution and partial characterization).[20] The preparation of DPD from an in vitro two-step enzymatic conversion[7] of *S*-adenosylhomocysteine (SAH) mediated by the enzymes Pfs and LuxS yields a DPD solution that is concentration limited by the low solubility of SAH and contains the byproducts adenine and homocysteine in stoichiometric amounts. Here we report studies on the complexation of hydrated DPD (**2**) and a natural analogue, laurencione (**5**), with borate, which suggest a high affinity in this interaction and provide a technique for purification of DPD/AI-2 through affinity column chromatography.

As a ^{11}B chemical shift reference, borate complexes of *cis*-cyclopentane-1,2-diol were prepared by azeotropic removal of water from a mixture of *cis*-cyclopentane-1,2-diol and boric acid in $CDCl_3$. The product mixture showed peaks at δ 9.39 and 7.65 ppm in the ^{11}B NMR spectrum that were assigned to the 2:1 and 1:1 diol/borate complexes, respectively.[21] Spontaneous formation of the same borate complexes was observed by ^{11}B NMR from *cis*-cyclopentane-1,2-diol in a medium of saturated $NaHCO_3$ and saturated $B(OH)_3$. D-Ribose and borate in the same medium gave complexes with ^{11}B NMR chemical shifts (δ 11.48 and 6.71), consistent with the 2:1 and 1:1 sugar/borate complexes.

Laurencione, **5**, from the marine sponge *Laurencia spectabilis*, differs from DPD only by the absence of the C-4 OH group.[22] It can be prepared by direct SeO_2 oxidation of 4-hydroxy-2-pentanone but is unstable when concentrated;[23] we note that it forms an intractable orange solid in organic or aqueous solution when concentrated above 0.1 M. This is reminiscent of the enzymatic preparations of DPD that also form an orange precipitate with loss of biological activity upon lyophilization.

Laurencione forms stable borate complexes in aqueous media at pH 7.8, presumably through the cyclic form (**8**) and the hydrate, **9**. When **5** is titrated into a solution of D_2O saturated in $NaHCO_3$ and $B(OH)_3$ (pH 7.8), the ^{11}B NMR shows the excess borate peak (19.17 ppm) and a new peak at δ 5.94 ppm, which we assign to the 1:1 borate complex, **6** (Figure 2a,b).[24] As more **5** is added, a second peak becomes

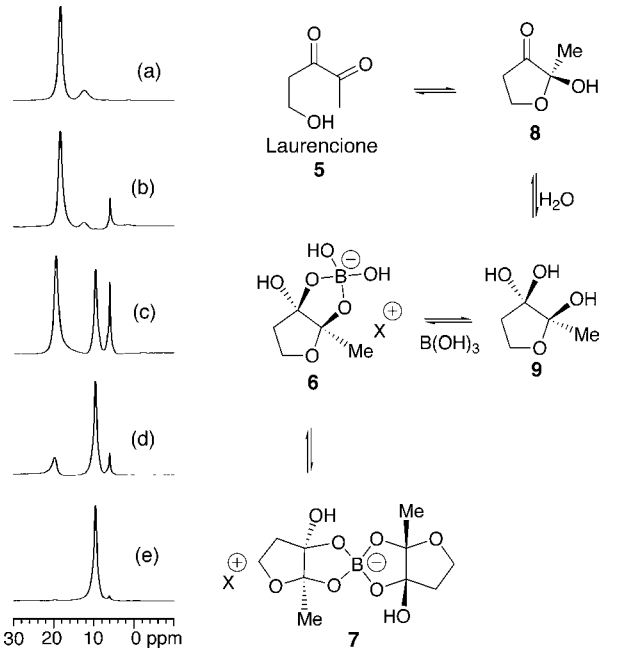

Figure 2.

predominant (δ 9.48, Figure 2c,d) and is assigned to the 2:1 complex, **7**. With a molar ratio of **5**:borate of approximately 2:1, the borate peak disappears and only the peak at δ 9.48 remains (Figure 2e). The instability of **5** makes precise determination of the equilibrium constant difficult, although the results suggest that complex formation is highly favor-

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able. The structure of dimeric complex **7** is supported by mass spectral data.[24]

The high affinity of **9** for boron can be used to catch and release laurencione from aminophenylboronic acid immobilized on polyacrylamide beads (Figure 3).[25]

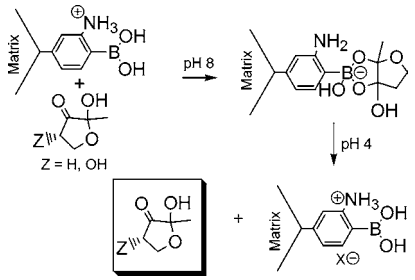


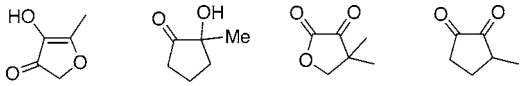
Figure 3. Catch-and-release with a boric acid resin.

This simple procedure involves stirring the beads (100 μmol boronate/mL gel) with **5** in H_2O at pH 7.8, followed by washing with saturated $NaHCO_3$ and release with 10−20% formic acid. The acidic filtrate from the beads is collected in a tube containing solid $NaHCO_3$ to ensure that the solution is quickly buffered at pH 7.8. Addition of boric acid to the collection tube generates the borates **6** and **7**. Since the binding and release are fast, prolonged incubation of DPD or laurencione with the polymer-supported boronate was unnecessary.

This technology can be applied as a procedure for purifying DPD from the enzymatic synthesis. The broth[7] is passed over the boronate resin in an aqueous $NaHCO_3$ medium. After the beads are washed, the DPD is released from the solid phase with 20% aq formic acid, reversing complexation and yielding a concentrated and purified solution of DPD (pH at 7.8). The eluant containing DPD can be stabilized by addition of borate to form *V. harveyi* AI-2. This preparation showed a biological activity increase of >600-fold over the enzymatic preparation. Attempts to concentrate the enzymatic preparations via lyophilization led to an orange intractable precipitate, analogous to that formed upon concentration of laurencione.

The spontaneous hydration of **1**[26] and **5** may be critical for the binding to boron and, in turn, the biological activity. Presumably, the pattern of oxygen substituents in **1** and **5** contributes to the ease of hydration. The activity of laurencione in the *V. harveyi* bioassay was 100-fold less than that of enzymatically prepared DPD; however, the activity of laurencione in the assay may have been masked since this molecule also inhibits cell growth.

MHF (**4**) does not show boron complexation but has activity comparable to laurencione in the bioassay. Ribose and analogue **10** were found to bind boron but were inactive (<0.001% of the activity of DPD) in the *V. harveyi* assay. Analogues **11** and **12** show neither significant boron complexation nor biological activity.[27]



In summary, the affinity for borate of analogues of the bacterial quorum sensing signal, DPD/*V. harveyi* AI-2, was tested. In particular, the dehydroxy analogue, laurencione (**5**), shows a high affinity for boron through spontaneous cyclization, hydration, and then borate complexation. Catch-and-release with a borate-bearing resin can be used to provide the first purification procedure for DPD from the biosynthetic broth, giving samples with a 600-fold increase in activity.

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Supporting Information Available: Procedures for the borate binding studies by ^{11}B NMR, including the titration experiment with laurencione. This material is available free of charge via the Internet at http://pubs.acs.org.

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(27) Full description of the structure−activity profile of a collection of related analogues is in preparation.