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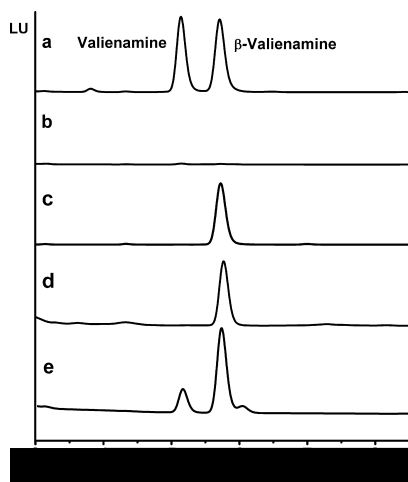


Figure 3. ^1H NMR spectra of Valienamine (A) and β -Valienamine (B) in CDCl₃. The x-axis represents chemical shift in ppm, ranging from 0 to 10. The y-axis represents intensity. Spectrum (a) shows the full spectrum with peaks at approximately 4.16 ppm (Valienamine) and 3.27 ppm (β -Valienamine). Spectra (b), (c), (d), and (e) show the same peaks with varying relative intensities, indicating different ratios of the two isomers.

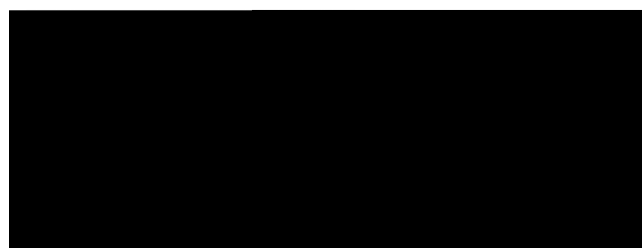
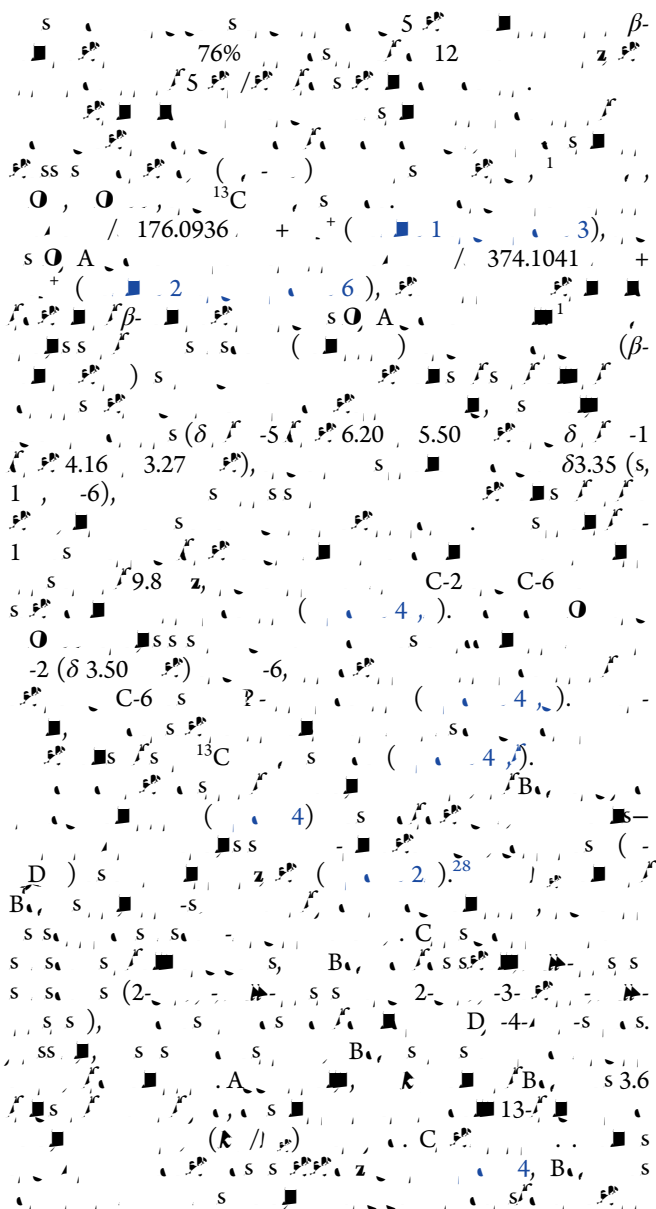
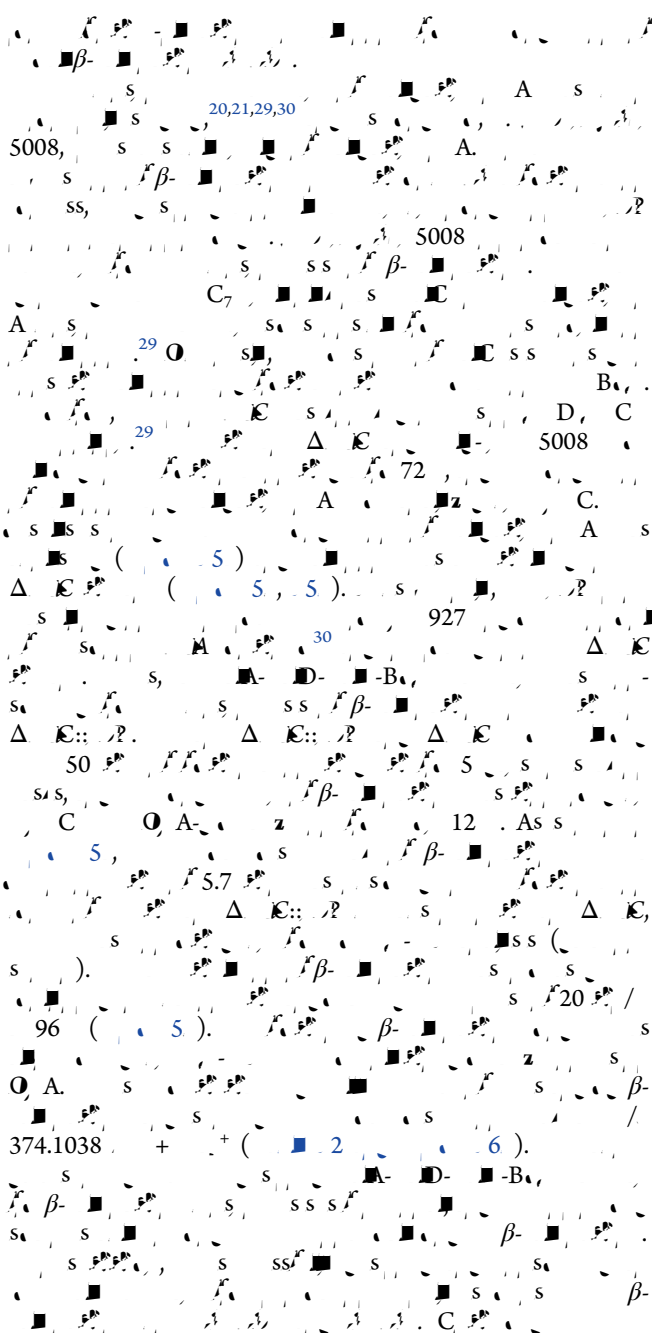


Figure 4. ^{13}C NMR spectra of Valienamine (A) and β -Valienamine (B) in CDCl₃. The x-axis represents chemical shift in ppm, ranging from 0 to 100. The y-axis represents intensity. Spectrum (a) shows the full spectrum with peaks at approximately 176.0936 ppm (Valienamine) and 374.1041 ppm (β -Valienamine). Spectra (b), (c), (d), and (e) show the same peaks with varying relative intensities, indicating different ratios of the two isomers.



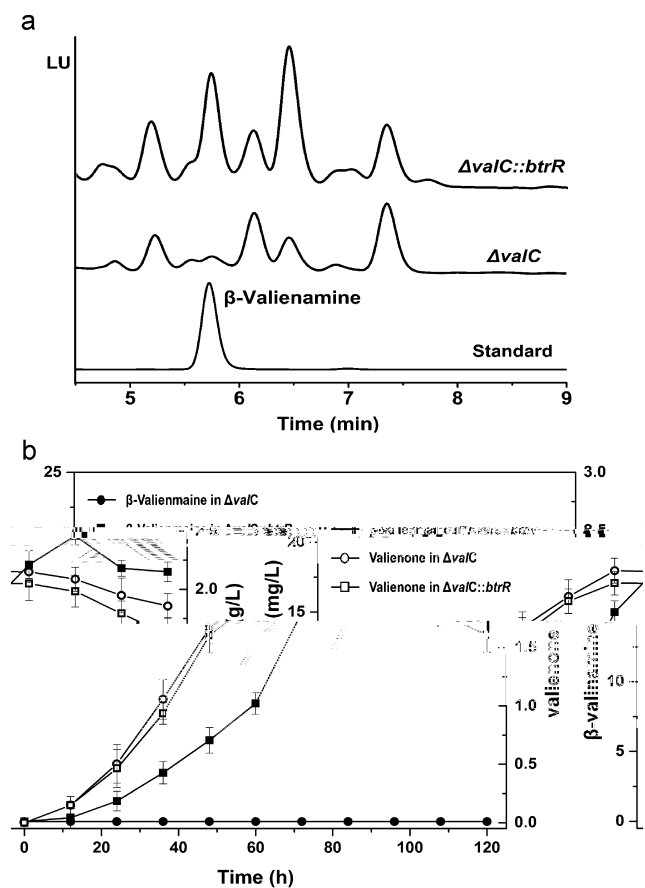


Figure 5. β -Valienamine production in $\Delta valC$ and $\Delta valC::btrR$ strains. (a) HPLC chromatograms of β -Valienamine in $\Delta valC$ and $\Delta valC::btrR$ strains. (b) Growth curves of β -Valienamine and Valienone production in $\Delta valC$ and $\Delta valC::btrR$ strains.

MATERIALS AND METHODS

Chemicals. β -Valienamine, Valienone, and other reagents were purchased from Sigma-Aldrich. β -Valienamine was purified from the reaction mixture and the fermentation broth of $\Delta valC::btrR$ as described in the text.

Molecular Phylogenetic Analysis by Maximum Likelihood. The DNA sequences of the β -Valienamine synthase genes from *Streptomyces* and *Actinomyces* were aligned using ClustalW. The phylogenetic tree was constructed using the Maximum Likelihood method with the GTR+I+G4 model in PhyML. The support values for the nodes were calculated using the Bootstrap method (1000 replicates).

β -Valienamine was purified from the reaction mixture and the fermentation broth of $\Delta valC::btrR$ as described in the text.

Expression and Purification of SATs.

Recombinant SATs were expressed in *Escherichia coli* BL21 (DE3) cells. The cells were grown in LB medium containing 100 μ M IPTG at 37 $^{\circ}$ C. The cell lysate was centrifuged at 10,000 rpm for 10 min. The supernatant was filtered through a 0.45 μ m filter and then dialyzed into storage buffer. The dialysate was concentrated by ultrafiltration and then stored at -20 $^{\circ}$ C. The concentration of the purified SATs was determined by SDS-PAGE and densitometry.

Enzymatic Activity and the Kinetic Parameter Assays.

The enzymatic activity of SATs was measured by the formation of β -Valienamine from Valienone. The reaction mixture contained 100 μ M Valienone, 100 μ M NADP⁺, and 100 μ M NADPH in 50 mM Tris-HCl buffer (pH 7.5) at 37 $^{\circ}$ C. The reaction was initiated by the addition of SATs. The reaction was stopped by the addition of 10% TCA. The amount of β -Valienamine was determined by HPLC. The kinetic parameters K_m and V_{max} were determined by Lineweaver-Burk plots.

Stereopurity Determination of β -Valienamine by HPLC with Precolumn Derivatization Using OPA.

The stereopurity of β -Valienamine was determined by HPLC with precolumn derivatization using OPA. The reaction mixture was extracted with ethyl acetate and then dried. The residue was dissolved in 100 μ L of 0.1 M NaOH. The solution was then derivatized with OPA. The derivatized sample was injected into a HPLC system equipped with a DB-C18 column (5 μ m, 4.6 \times 150 mm) and a fluorescence detector. The elution was monitored at 340 nm excitation and 445 nm emission. The retention time of β -Valienamine was 5.7 min.

β -Valienamine Purification from the Reaction Mixture and the Fermentation Broth of $\Delta valC::btrR$.

β -Valienamine was purified from the reaction mixture and the fermentation broth of $\Delta valC::btrR$ as described in the text. The reaction mixture was extracted with ethyl acetate and then dried. The residue was dissolved in 100 μ L of 0.1 M NaOH. The solution was then derivatized with OPA. The derivatized sample was injected into a HPLC system equipped with a DB-C18 column (5 μ m, 4.6 \times 150 mm) and a fluorescence detector. The elution was monitored at 340 nm excitation and 445 nm emission. The retention time of β -Valienamine was 5.7 min.

High Resolution-Mass Spectrometry (HR-MS) and Nuclear Magnetic Resonance Spectrometry (NMR).

HR-MS and NMR were used to identify and confirm the structure of β -Valienamine. The molecular weight of β -Valienamine was determined by HR-MS. The structure was confirmed by NMR. The ¹³C NMR spectrum of β -Valienamine was recorded in D₂O. The chemical shifts of the ¹³C NMR spectrum were in the range of 129.0–62.3 ppm.

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