Convergent Synthesis of trans-2,6-Disubstituted Piperidine Alkaloid, (−)-iso-6-Spectaline by Palladium-Catalyzed Cyclization

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The plant alkaloids, iso-6-spectaline and spectaline, isolated from the Cassia or Senna genera contain a characteristic 2,6-disubstituted piperidin-3-ol scaffold. Although both natural products are reported to exhibit a variety of interesting biological activities, few stereo-selective schemes for the construction of the 2,6-disubstituted scaffold have been reported. Following our previous studies regarding the synthesis of (−)-spectaline, herein we report the first convergent synthesis of (−)-iso-6-spectaline using a cross-metathesis under thermal conditions where the cis-2,6-disubstituted piperidin-3-ol scaffold is condensed with a long alkyl chain containing a terminal olefin. The cis-2,6-disubstituted piperidin-3-ol used in the synthesis was prepared simply via Pd(II)-catalyzed diastereoselective cyclization. It was confirmed that (+)-spectaline, an epimer of (−)-iso-6-spectaline, was selectively synthesized by the cross-metathesis reaction under less intense thermal conditions starting from the same cis-2,6-disubstituted piperidin-3-ol derivative.

Key words spectaline; iso-6-spectaline; convergent synthesis; alkaloid; Pd(II)-catalyzed cyclization

Introduction

The alkaloids iso-6-spectaline (1) and spectaline (2), typically isolated from the genera Cassia or Senna, contain a characteristic 2,6-disubstituted piperidin-3-ol scaffold (Fig. 1). These alkaloids are reported to exert a variety of biological effects including DNA-modifying, antibacterial, anti-candidal, and HepG2 cell anti-proliferative activities. In addition, they have been reported to inhibit the growth of human-infective Trypanosoma species and the activity of acetylcholinesterase; however the structure–activity relationship, especially for the stereo-structures of the substituents on the piperidine scaffold, has not been examined.

Synthetic studies of the iso-6-spectaline-containing trans-2,6-disubstituted piperidin-3-ol scaffold have not been reported to date. In contrast, several early synthetic studies of (+)-spectaline with a cis-2,6-disubstituted piperidin-3-ol scaffold were achieved stereoselectively although some refinement regarding the reaction steps and/or the convergence is required to proceed with the structure–activity studies. A recent synthesis of (−)-spectaline from a chiral aziridine was achieved based on a linear synthetic scheme. In the syntheses of piperidine alkaloids isolated from Calvia ladybird beetles, Pd(II)/Cu(II)-catalyzed aminocyclization-methoxycarbonylation was used to construct the trans-2,6-disubstituted piperidin-3-ol scaffold. However, the product was a mixture of trans- and cis-2,6-disubstituted piperidin-3-ol in a 3:1 ratio.

In 2015, we reported a convergent synthesis of (+)-spectaline by coupling a long alkyl chain with the cis-2,6-disubstituted piperidin-3-ol scaffold constructed by Pd(II)-catalyzed diastereoselective cyclization. In another study regarding (+)-spectaline, we reported the possibility of constructing the trans-2,6-disubstituted piperidin-3-ol scaffold. In Pd(II)-catalyzed cyclization, a precursor compound containing bulky hydroxyl-protecting groups afforded a trans-2,6-disubstituted piperidin-3-ol derivative, although the yield was rather low. In the subsequent synthetic studies involving the trans-2,6-disubstituted piperidin-3-ol alkaloids, it was revealed that the cis-2,6- or trans-2,6-disubstituted piperidin-3-ol scaffold can be selectively prepared by changing the reaction mode of the cross metathesis reaction, a key step in the convergent synthetic scheme.

Results and Discussion

First, to improve the yield of the trans-2,6-disubstituted piperidin-3-ol derivative obtained from the bulky precursor in the Pd(II)-catalyzed cyclization, a linear precursor, containing an unprotected hydroxyl group combined with a bulkier tert-butyl diphenylsilyl (TBDPS) group was treated with PdCl₂ (0.1 equiv.) at 25° (Chart 1). The unprotected hydroxyl group facilitated the yield improvement with the bulkier TBDPS (compared to tert-butyl dimethylsilyl (TBDMS) employed in our previous synthesis), which was selected to direct the reaction to yield the desired trans-2,6-disubstituted...
piperidine-3-ol scaffold. The cyclized product, 4, was obtained as a single diastereomer\(^{21}\) with an improved yield compared to that of the previous synthesis, since the precursor 3 contains an un-protected primary alcohol. However, the stereostructure of 4 could not be identified due to the lack of a clear nuclear Overhauser effect (NOE) signal. In addition, following the introduction of an alkyl substituent at 6-position via cross-metathesis followed by deprotection of the TBDPS and tert-butoxycarbonyl (Boc) groups, the obtained product 6 was a mixture of cis-2,6- and trans-2,6-disubstituted piperidin-3-ol alkaloids, suggesting a possibility of epimerization during the metathesis and/or thermal tetrabutylammonium fluoride (TBAF) reaction due to the presence of the olefin functional group.

Based on the above results, to identify the stereostructure of 4 and examine the possibility of stereo-conversion during the metathesis reaction, the cross-metathesis product was converted to spectaline or iso-6-spectaline according to the route shown in Chart 2. In this scheme, the olefin functional group in metathesis product 8 is first reduced to avoid possible isomerization under the subsequent thermal reaction conditions due to the olefin's functionality. Thus, precursor 3 was treated with PdCl\(_2\) in tetrahydrofuran (THF) at 25\(^\circ\) as above to afford the cyclized product, 7, as a single diastereomer (Chart 2). Although the absolute structure of 7 could not be confirmed, its \(^1\)H-NMR spectrum was exactly the same as that of 4. Compound 7 was then condensed with tetradec-13-ene-2-one via cross-metathesis using the same reaction conditions (in CH\(_2\)Cl\(_2\), reflux) employed in our previous synthesis.\(^{13}\) The resulting compound 8 was obtained as a single \(E\)-isomer, which was verified to be due to the TBDPS group at 3-position in 7.\(^{22}\) Product 8 was subsequently reduced by catalytic hydrogenation, and the reduced product was treated with TBAF in THF (reflux) followed by acid treatment to deprotect the Boc group. The resulting final product showed an identical \(^1\)H-NMR spectrum to the previously synthesized (+)-spectaline, which strongly suggests that intermediates 7 and 8 contain the cis-2, 6-disubstituted piperidin-3-ol scaffold.
These results combined with those obtained in our previous synthesis of (+)-spectaline confirm that the isomerization at 6-position of the spectaline scaffold does not occur under a specific metathesis condition (in CH$_2$Cl$_2$, reflux) nor in the following hydrogenation and acidic conditions.

Next, to evaluate the possibility of isomerization from the cis-2,6-disubstituted piperidin-3-ol scaffold to trans-2,6-disubstituted piperidin-3-ol, the cyclized cis-2,6-disubstituted product 7 was reacted with tetradec-13-ene-2-one by the cross-metathesis reaction under specific thermal conditions (Chart 3). The product was then reduced by catalytic hydrogenation followed by treatment with TBAF and HCl as shown in Chart 2. The final product yielded a significantly different NMR spectra compared to those of the (+)-spectaline synthesis as described above. Instead, the spectra showed good agreement with those reported for natural iso-6-spectaline, clearly indicating that a cis-2,6- or trans-2,6-disubstituted piperidin-3-ol scaffold can be selectively constructed from 7 using specific cross-metathesis reaction conditions. Thus, a total synthesis of (−)-iso-6-spectaline 1 was achieved by combining a Pd(II)-catalyzed cyclization with a cross-metathesis reaction under specific thermal conditions.

The energetics of the 2,6-cis form of 8, calculated using the SPARTAN’16 program, suggests that the stable conformation is likely the chair-form, i.e., 11 (2-CH$_3$-ax., 3-OTBDPS-equ., 6-alkyl-ax.), which is more stable than 10 (2-CH$_3$-eq., 3-OTBDPS-ax., 6-alkyl-equ.) with an energy value of 350.15 kcal/mol (Fig. 2). Similarly, the energetics of the 2, 6-trans form of 9, suggest that the expected stable conformation is 13 (2-CH$_3$-ax., 3-OTBDPS-equ., 6-alkyl-equ.), which is more stable than 14 (2-CH$_3$-eq., 3-OTBDPS-ax., 6-alkyl-ax.) with an energy value of 71.26 kcal/mol. In addition, the cis-conformation of 11 is expected to be more stable than the trans-conformation of 13 by 3.12 kcal/mol, resulting in preferential production of a cis-form 8 at lower-temperature metathesis (40°). In contrast, the cross-metathesis reaction at 80° resulted in an isomerization via intermediate 12. A proton transfer-reaction via 12 occurs dominantly from the less hindered upside of 12 to preferentially yield 13 as a 2, 6-trans product. Since the isomerization would not proceed in the absence of the olefin structure, the H$_2$/Pd reduced product obtained from 8 or 9 maintained its corresponding cis or trans configuration following the deprotection of TBDPS and Boc groups to yield (+)-spectaline or (−)-iso-6-spectaline. Therefore, spectaline or iso-6-spectaline can be selectively synthesized starting from the same cyclized precursor with a cis-2,6-piperidin-3-ol scaffold.

The antimicrobial activity of the synthesized (−)-iso-6-spectaline and (+)-spectaline was evaluated in vitro against Staphylococcus epidermidis. The prepared (−)-iso-6-spectaline exhibited an antimicrobial activity with an minimal
inhibitory concentration (MIC) value of 100 µg/mL, whereas that of (−)-spectaline was 12.5 µg/mL. From this parallel comparison using synthetic compounds, it was demonstrated that a single configuration conversion at 6-position of the piperidine scaffold affects the biological behavior of alkaloids containing a characteristic 2,6-disubstituted piperidin-3-ol scaffold.

Conclusion
The first convergent synthesis of (−)-iso-6-spectaline was achieved using a cross-metathesis reaction under specific thermal conditions, where a cis-2,6-disubstituted piperidin-3-ol derivative prepared by Pd(II)-catalyzed diastereoselective cyclization was coupled with an alkyl olefin side chain. Starting from the same cis-2,6-disubstituted piperidin-3-ol derivative, (+)-spectaline, an epimer of (−)-iso-6-spectaline, was also synthesized via the cross-metathesis reaction at a lower temperature. Thus, we have demonstrated that the trans- or cis-2,6-disubstituted piperidin-3-ol scaffold of natural products can be selectively constructed starting from the same cis-precursor by modifying the cross-metathesis reaction conditions. This synthetic scheme can easily be applied for convergent syntheses of a variety of 2,6-disubstituted piperidin-3-ol alkaloids to enable the performance of structure–activity relationship studies of these biologically interesting natural products.

Experimental
General Methods for Chemistry
The melting point of each compound was determined using a Yanaco apparatus and was uncorrected. Analytical TLC was performed using a silica gel (Silica gel 70 F254 plate, 0.25 mm TLC Plate-Wako). Column chromatography was performed using Wakogel® 60N (particle size, 63–212 µm) and the 1H- and 13C-NMR spectra were measured using a Bruker AV-300 FT-NMR spectrometer. Mass spectra were obtained using a LCMS-IT-TOF mass spectrometer. Optical rotations were determined using a HORIBA SEPA-300 polarimeter.

(2S,3S,5S)-N-(tert-Butyloxycarbonyl)-3-(tert-butyldiphenylsilyloxy)-2-methyl-6-vinylpiperidin-3-ol tetradec-13-en-2-one (7)
A solution of 3 (16 mg, 0.032 mmol) in THF (1.0 mL) was treated with PdCl2 (0.6 mg, 0.0034 mmol) under an argon atmosphere. After stirring for 1 h at 25 °C, the reaction mixture was filtered and concentrated. The residue was purified via silica gel column chromatography (hexane/EtOAc = 20:1) yielding 7 (11 mg, 72%) as a colorless oil. 1H-NMR spectra of the isolated product showed the product was a mixture of two diastereomers (see Supplementary Materials). 1H-NMR δ: 1.16 (2H, d, J = 6.6 Hz), 1.25–1.40 (20H, m), 1.46–1.58 (5H, m), 1.92–1.98 (2H, m), 2.14 (3H, s), 2.42 (2H, t, J = 7.4 Hz), 2.68 (0.7H, m), 2.84–2.87 (0.3H, m), 2.91–2.94 (0.7H, m), 3.15–3.17 (0.3H, m), 3.58 (0.3H, brs), 3.66 (0.7H, brs).

(+)-Spectraline (2)
A solution of 3 (31 mg, 0.065 mmol) and tetradec-13-en-2-one (82 mg, 0.39 mmol) in CH2Cl2 (5.0 mL), Grubbs 2nd catalyst (8.3 mg, 0.0098 mmol) was added. After stirring for 1 h under reflux, an additional solution of Grubbs 2nd catalyst in CH2Cl2, (8.3 mg, 0.0098 mmol) was added, and after an additional stirring for 1 h, Grubbs 2nd catalyst in CH2Cl2, (8.3 mg, 0.0098 mmol) was further added. After stirring for 1 h under reflux, the solvent was removed under reduced pressure and the residue was roughly purified via silica gel column chromatography (hexane/EtOAc = 10:1). The product was then used for the next step without further purification by dissolving in CH2Cl2 (1 mL) and adding Pd-C (5.5 mg). After stirring for 18 h under a/h2 atmosphere, the reaction mixture was filtered and concentrated. The crude product was dissolved in THF (5 mL) and TBAF (1.0 mol/L, 0.7 mL) was added. After stirring for 16 h under reflux, the reaction was quenched with saturated aqueous Na2SO4, and dried over Na2SO4, and concentrated. The residue was purified via silica gel column chromatography (hexane/EtOAc = 2:1) yielding 2 as a yellowish oil (6.1 mg, 29%, 4 steps). The product showed an identical NMR spectrum as that of the above-mentioned compound 4 and our previous report.31)
CH₃Cl₂ (5.0 mL), Grubbs 2nd catalyst (8.5 mg, 0.010 mmol) was added. After stirring for 1 d under reflux, an additional solution of Grubbs 2nd catalyst in CH₃Cl₂ (8.5 mg, 0.010 mmol) was added. After stirring for an additional 1 d under reflux, Grubbs 2nd catalyst in CH₃Cl₂ (8.5 mg, 0.010 mmol) was added and the mixture was stirred for 1 d under reflux. After stirring for 1 d under reflux, the solvent was removed and the residue was heated at 80° for 1 d. The product 9 was treated with H₂-Pd/C, TBAF, and HCl as described above. The final product was washed with hexane to yield 1 as a colorless solid (3.3 mg, 15%, 4 steps). mp 121.5–123.0°; [α]D²⁰ = −2.6 (c 0.17, CH₃Cl₂); ¹H-NMR δ: 1.26–1.39 (20H, m), 1.55–2.14 (9H, m), 2.17 (3H, s), 2.42 (2H, t, J = 7.4 Hz), 2.97 (1H, m), 3.21–3.25 (1H, m), 3.89 (1H, brs). ¹³C-NMR δ: 15.8, 21.9, 23.9, 25.5, 29.4, 29.5, 30.5, 33.2, 43.8, 57.1, 58.4, 66.3, 209.6; high resolution-electrospray ionization (HR-ESI)-MS calcd for C₁₃H₂₄NO₂ [M + H]+ 326.0354, found 326.0351 m/z.

Equilibrium Geometry Optimization and Energy Calculations The in silico experiments were performed using the SPARTAN’16 program (Wavefunction, Inc.) installed on a Dell Vostro system running on an Intel Corei7 with a Windows 7 operating system. The equilibrium geometry of the piperidine analogues (cis- and trans-forms; 8 and 9, respectively) in the gas phase ground state was calculated by density functional theory (DFT) using theωB97X-D method. The stable conformations of the cis-8 and trans-9 isomers were given both as chair-like forms with energy values of −2244.98485 and −2244.976507 hartrees (1 hartree = 627.510 kcal/mol), respectively.

The energies of the two chair-like conformers of the cis- (10 and 11) and the trans-forms (13 and 14) in the gas phase ground state were also calculated by DFT using theωB97X-D method. The energy of the 2,6-cis-form 11 containing a 3-OTBDPS group at the equatorial position was −2244.981725 hartrees, and that of the cis-form 10 containing 3-OTBDPS group at an axial position was −2244.422245 hartrees. The energy of the 2,6-trans-form 13 containing a 3-OTBDPS group at an equatorial position was −2244.976725 hartrees, and that of the trans-form 14 containing the 3-OTBDPS group at an axial position was −2245.863165 hartrees.

Antimicrobial Assay The MIC of (−)-iso-6-spectaline and (+)-spectaline were determined with broth by the typical twofold serial dilution method.¹² The growth media for S. epidermidis (NBRC 100911) was Difco™ Mueller Hinton broth. Before starting the MIC assay, the microbical cells were grown for 24 h and suspended in broth at a concentration of 1.5 × 10⁶ cells/mL. The concentration of the compounds started with 200 µg/L in a medium containing 1.0% dimethyl sulfoxide (DMSO), and underwent twofold serial dilution. Then, 100 µL of the solution was poured into a 96-well microtiter plate and inoculated with microbial suspensions of 7.5 × 10⁴ cells. The MIC was defined as the lowest concentration of antimicrobial agent that inhibited the development of visible growth after 24 h of incubation at 37°.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

References and Notes