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Asymmetric Total Synthesis of (−)-VM55599: Establishment of the Absolute Stereochemistry and Biogenetic Implications

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Abstract: The first asymmetric biomimetic total synthesis of VM55599 (**13**) has been achieved utilizing an intramolecular Diels−Alder cycloaddition as a key step. The synthetic material was utilized to elucidate the absolute stereochemistry of the natural product. The results are discussed in terms of a unified biogenesis of the paraherquamides and VM55599.

Introduction

The paraherquamides[1] (Figure 1), together with the brevianamides,[2] marcfortines,[3] and sclerotamides[4] are secondary metabolites of fungal origin that feature a common bicyclo[2.2.2]-diazaoctane core. It has been postulated that this ring system is generated through an intramolecular Diels−Alder cycloaddition of the C_5 moiety across the α-carbons of the amino acid subunits, as depicted in Scheme 1.[5]

Everett and co-workers described in 1993 the isolation of VM55599 (**13**, Figure 1), a minor metabolite from culture extracts of a *Penicillium* sp. (IMI332995) that also produces paraherquamide A (**1**), among other paraherquamides.[6] Taking into account the structural similarities between these co-occurring metabolites, these authors proposed that VM55599 (**13**) might indeed be a biosynthetic precursor of paraherquamide A (**1**). The relative stereochemistry of VM55599 (**13**) was assigned by Everett and co-workers through extensive ^{1}H NMR nOe experiments, but the small quantity of this compound that was isolated precluded the determination of its absolute configuration.

An important detail about the absolute configuration that these authors hypothesized for VM55599 (**13**) involves the relative

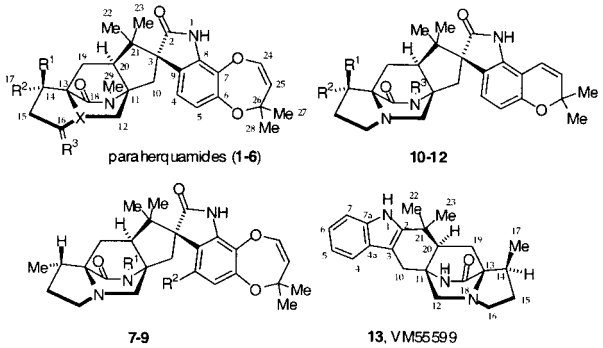
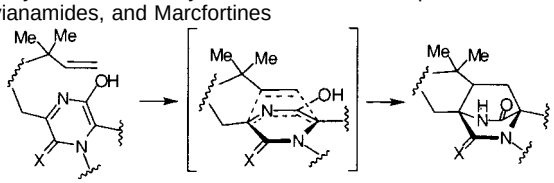


Figure 1. **1**, Paraherquamide A, R^1 = OH, R^2 = Me, R^3 = H_2, X = N; **2**, paraherquamide B, R^1 = H, R^2 = H, R^3 = H_2, X = N; **3**, paraherquamide C, R^1 = R^2 = CH_2, R^3 = H_2, X = N; **4**, paraherquamide D, R^1 = O, R^2 = CH_2, R^3 = H_2, X = N; **5**, VM55596, R^1 = OH, R^2 = Me, R^3 = H_2, X = N^+−O^-; **6**, VM55597, R^1 = OH, R^2 = Me, R^3 = O, X = N; **7**, paraherquamide E (VM54159), R^1 = Me, R^2 = H; **8**, SB203105, R^1 = Me, R^2 = OH; **9**, SB200437, R^1 = H, R^2 = H; **10**, paraherquamide F (VM55594), R^1 = H, R^2 = Me, R^3 = Me; **11**, paraherquamide G (VM54158), R^1 = OH, R^2 = Me, R^3 = Me; **12**, VM55595, R^1 = H, R^2 = Me, R^3 = H.

Scheme 1. Proposed Formation of the Bicyclo[2.2.2]diazaoctane Ring System in the Biosynthesis of the Paraherquamides, Brevianamides, and Marcfortines



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configuration at C-14 that disposes the C-17 methyl residue *syn*- to the bridging C_5 moiety. In stark contrast, in all the other paraherquamides with a methyl group in an equivalent position the methyl group is disposed *anti*- to the bridging C_5 moiety. Therefore, if VM55599 (**13**) were indeed a biosynthetic precursor to the paraherquamides as proposed, the configuration at C-14 would have to be inverted at some point along the biosynthetic pathway.

Previous studies from our laboratories have shown that (*S*)-isoleucine (L-Ile) serves as the precursor of the β-methyl-β-hydroxyproline ring in paraherquamide A (**1**). This mandates that the L-Ile side chain stereochemistry is retained at C-14 in paraherquamides **7**−**10**, **12** and that hydroxylation in paraherquamides **1**, **5**, **6**, and **11** occurs with *net retention* at C-14.[7] Accordingly, these results brought into question the capacity of VM55599 (**13**) to serve as a biosynthetic precursor to the paraherquamides. If, as one could reasonably speculate, L-Ile is not only a biosynthetic precursor to the paraherquamides, but also to VM55599 (**13**), the absolute stereochemistry of this compound must be that depicted in Figure 1. Thus, the absolute configuration of the bicyclo[2.2.2]diazaoctane ring system of VM55599 would be anticipated to be enantiomorphic to that of all other members of the paraherquamide family.

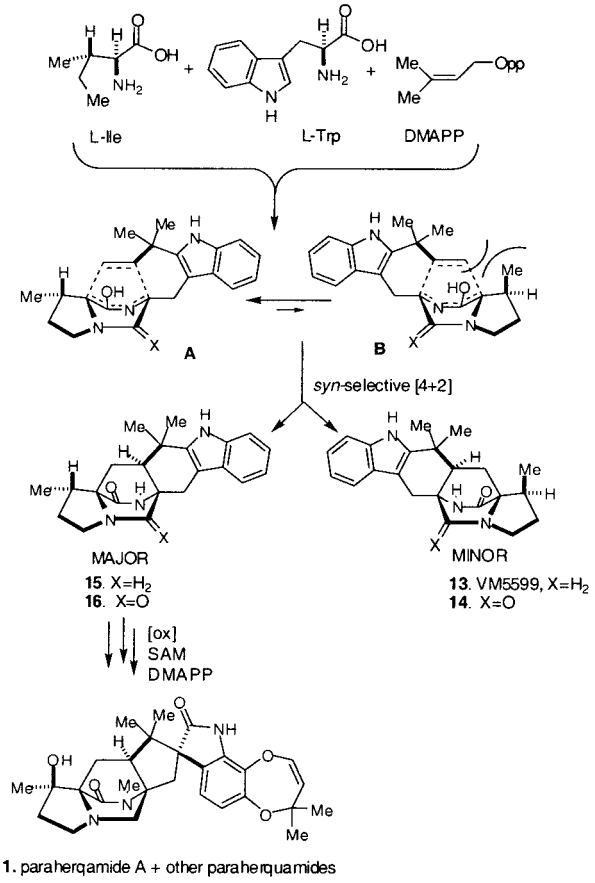
These experimental observations led us to propose a unified biosynthesis of the paraherquamides and VM55599 (**13**), as shown in Scheme 2.[8] In this proposal, the biosynthetic precursors of the paraherquamides and that of VM55599 would arise as diastereomeric products of the Diels−Alder cycloaddition of a common azadiene through two of four possible diastereomeric transition states.

The minor product of this cycloaddition culminating in VM55599 would constitute approach of the dienophile *syn*- to the methyl group (as in structure **B**, Scheme 2). The major product of the cycloaddition would be compound **15** or **16**, wherein the dienophile attacks the opposite face of the azadiene, *anti*- to the methyl group, which would give rise to the various paraherquamides.

This hypothesis was recently experimentally tested through feeding experiments of racemic, doubly ^{13}C-labeled compounds **13**−**16**.[9] These experiments revealed that only intermediate **15** was incorporated into paraherquamide A, while racemic, doubly ^{13}C-labeled VM55599 (**13**), **14**, and **16** were not incorporated. While these results lend support to the proposed biogenesis and provide a prediction as to the correct stereostructure of VM55599, the absolute stereochemistry of VM55599 remained uncertain, abrogating the connection of this metabolite to the paraherquamide pathway. Considering that VM55599 (**13**) is produced in very small quantities by the *Penicillium* sp. (>600:1 ratio of paraherquamide A:VM55599), a feeding experiment with labeled L-Ile proved impractical.

To determine the absolute configuration of VM55599 (**13**), we first resolved synthetic, racemic VM55599[8c,9] by chiral HPLC.[10] The two enantiomers readily separated, which were then individually compared to an authentic sample of natural VM55599. The first eluting enantiomer coincided in retention time and optical rotation with the natural VM55599 (**13**), and this procedure provided a way to prepare a few milligrams of the natural antipode of VM55599 (**13**). Both enantiomers were then transformed into their *N*-*p*-bromobenzoate derivatives through reaction with *p*-bromobenzoyl chloride and 2,6-lutidine in refluxing acetonitrile. However, these compounds failed to produce a monocrystal of sufficient quality for X-ray diffraction analysis in our hands. A plausible alternative involving an enantiospecific total synthesis starting with a chiral, nonracemic compound of known absolute configuration was subsequently investigated.

Scheme 2. Unified Biogenesis of the Paraherquamides and VM55599



Results and Discussion

In contemplating a stereochemically unambiguous asymmetric total synthesis in this general family, we envisioned installing the β-methylproline ring with the anticipated absolute stereochemistry in a manner in which the critical stereogenic center at C-14 would not be perturbed. Our recently reported racemic synthesis of VM55599 could not be adapted to suit this

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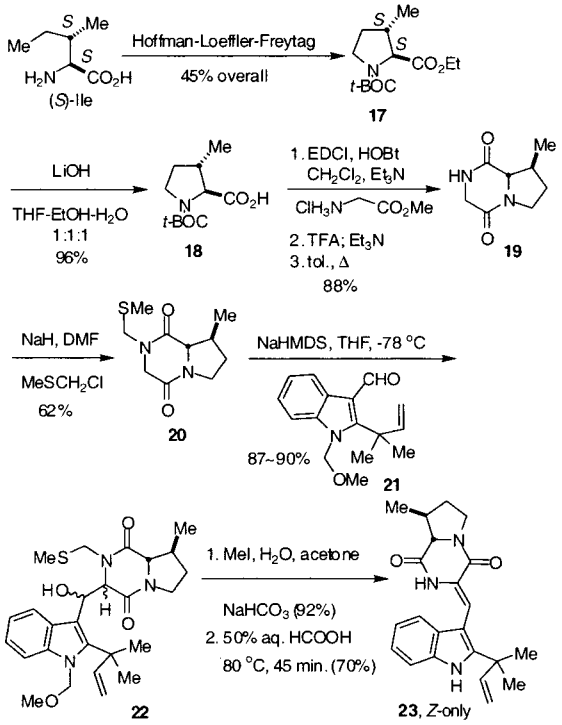
(10) The HPLC separation was carried out with an (*R*,*R*) Whelk-O2 10/100 1 cm diameter × 25 cm length semipreparative column. A mixture of heptane−*i*-PrOH, 82:18 was used as mobile phase. The detector was set at 220 nm. Racemic VM55599 (1 mg) was injected each time. Natural VM55599 had a retention time of 11.5 min, while *ent*-VM55599 was eluted at 14.9 min. The resolution was total (100% valley between peaks); this was confirmed later through re-injection of the isolated peaks under the same conditions, which showed no detectable amounts of the other enantiomer.

Scheme 3. Conversion of L-Ile into the Key Azadiene Precursor **23**

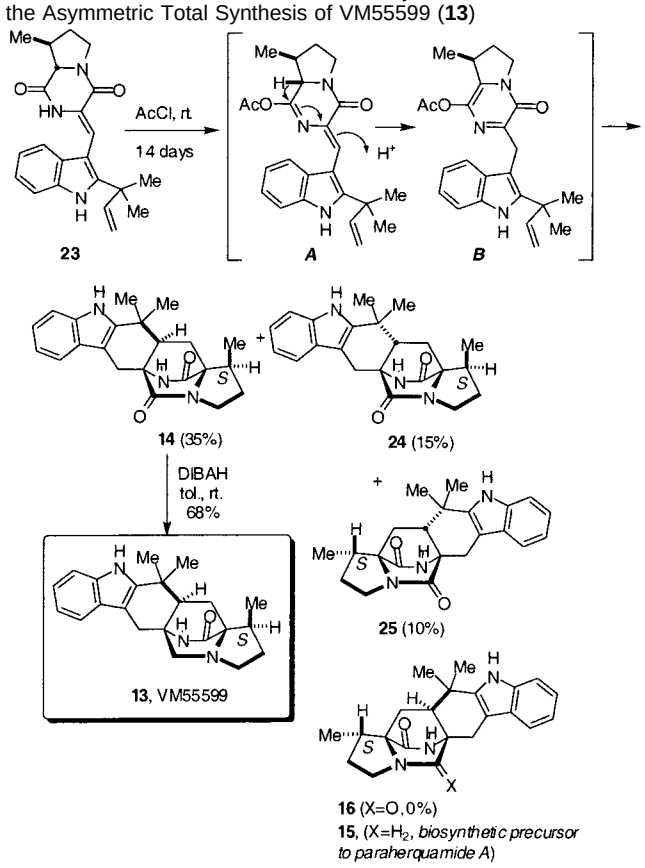


limitation since a dehydro-β-methylproline residue was employed as the azadiene precursor.[8c] It was readily recognized that, if a dehydrotryptophan residue could be employed in a similar manner, generation of the azadiene species should not perturb the β-position of the proline ring leading to optically pure cycloadducts. This strategy has been successfully realized as described below.

Inspired by Nature, we selected L-Ile as the starting material for the construction of the β-methylproline ring. The configuration of the carbon that supports the methyl group in L-Ile is *S*, and it can be transformed conveniently in five steps and 47% overall yield into the optically pure β-methylproline derivative **17** in multigram amounts using a Hoffman−Loeffler−Freytag sequence.[11] Compound **17** was easily hydrolyzed to acid **18** in 96% yield with LiOH in a mixture of THF−EtOH−H_2O 1:1:1. The acid **18** was then coupled to glycine methyl ester hydrochloride with EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) in the presence of 3-hydroxybenzotriazole and triethylamine in dichloromethane to give the corresponding dipeptide in 96% yield. The resulting crude compound was deprotected with TFA at room temperature. The crude deprotected dipeptide was treated with triethylamine to neutralize the excess TFA, and the mixture of salts thus prepared was heated in refluxing toluene to give the diketopiperazine **19** in 88% yield for three steps (Scheme 3).

The diketopiperazine **19** was protected as its *N*-methylthiomethyl (MTM) derivative through formation of the sodium salt with NaH in DMF at 0 °C, followed by reaction with chloromethyl methyl thioether at room temperature.[12] This reaction furnished a mixture of the MTM-protected diketopiperazine **20** (61.5% yield) and an epimer (10% yield), which were

Scheme 4. Intramolecular Diels−Alder Cycloaddition of **23** and the Asymmetric Total Synthesis of VM55599 (**13**)



easily separated by chromatography on silica gel. Although both compounds would have been potentially useful as substrates for the synthesis of **13**, only the major compound **20** was carried forward. After the enolate of **20** was generated through treatment with sodium bis(trimethylsilyl)amide in THF at −78 °C, condensation with aldehyde **21**[13] furnished a mixture of three diastereomers **22** in 87−90% combined yield. The relative stereochemistry of these compounds was not determined, but one of these isomers was separated for analytical identification purposes by means of column chromatography on silica gel. The MTM group in the mixture of diastereomers **22** was removed through treatment with an excess of iodomethane in acetone in the presence of an aqueous solution of $NaHCO_3$[12] to give a mixture of three diastereomers in 92% yield. Treatment of the diastereomeric mixture with 50% aqueous formic acid[13] at 80 °C for 45 min resulted in removal of the MTM group and dehydration of the alcohol, giving exclusively compound **23** in 70% yield. No trace of the corresponding *E*-isomer was detected. The configuration of the double bond was assigned through comparison of ^{1}H NMR spectral data with similar compounds.[14]

When compound **23** was treated with acetyl chloride at room temperature for 14 days according to conditions recently described by Liebscher and co-workers,[15] a mixture of three diastereomeric cycloadducts **14** (35% yield), **24** (15% yield), and **25** (10% yield) resulted (Scheme 4). It is assumed that acetylation of **23** yields the *O*-acyl lactim (**A**) that tautomerizes

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to azadiene **B**, which suffers intramolecular Diels−Alder cycloaddition from three of the four possible diastereomeric transition states, followed by loss of acetate. We did not detect the presence of any acetylated products or intermediates. The cycloadducts obtained had identical spectroscopic properties to the racemic compounds previously prepared in our laboratories.[8c,9] except for being optically pure.

Finally, reduction of the cycloadduct **14** with an excess of DIBAH in toluene at room temperature for 24 h gave synthetic (−)-VM55599 (**13**) that was identical in all respects to natural (−)-VM55599 (**13**), including optical rotation, CD and retention time on chiral HPLC (see Supporting Information). Thus, natural VM55599 (**13**) has the absolute configuration depicted (and predicted) in Scheme 1.

This result rigorously confirms the predicted absolute stereochemistry of VM55599, and provides additional experimental support for the unified biogenesis proposed for this compound and the paraherquamides (Scheme 2). It was quite surprising to observe that cycloadduct **16**, which contains the relative and absolute stereochemistry of the paraherquamides, was not detected from the cycloaddition reaction. The cycloadducts obtained in our racemic synthesis[8c] gave (as *O*-methyl lactim ethers), compounds stereochemically corresponding to **16**:**24**:**25**:**14** in a ratio 3.7:1.6:1:2.6. In the present case, the ratio is 3.5:1.5:1:0. Thus, both laboratory cycloaddition reactions[8c] display a proclivity for the formation of the VM55599 relative stereochemistry with differing proportions of the other three diastereomeric relationships. It is interesting to compare the *syn:anti-* ratios with respect to the relative stereochemistry at C-20.[16] In the present case, the *syn:anti-* ratio was 1.4:1. In our previously reported racemic system, the *syn:anti-* ratio was 2.4:1. The ratio of cycloaddition of the isoprene-derived dienophile across the azadiene with respect to the methyl group in the proline ring was 5:1, in the present case, favoring approach of the dienophile from the *same face* of the azadiene (see **B**, Scheme 2) as the methyl group and 1.47:1 in the racemic *O*-methyl lactim ether system.[8c] While structural differences in the laboratory azadienes (see **B**, Scheme 4) and that for the biological system (see **A**, Scheme 2) indeed exist, the intrinsic facial selectivity of this cycloaddition appears not to be greatly biased toward the paraherquamide stereochemistry. In the biological system, the diastereochemical distribution is expressed as >600:1[17] (corresponding to **15** or **16**:**13** or **14**) as evidenced by the complete lack of natural metabolites that would arise from substances containing the *anti*-stereochemistry[16] imbedded in either **24** or **25**.

Conclusions

This study has served to confirm the absolute stereochemistry of the putative shunt metabolite, VM55599, that is consistent with a unified biogenesis of the paraherquamides proposed earlier[8] and corrects a misassignment reported in the initial isolation and structure determination of this natural product.[6] It is now also clear that the absolute stereochemistry of the entire paraherquamide family arises from the single stereogenic center at the β-position of the β-methylproline moiety that is fashioned from the L-Ile side chain.[7,9] The laboratory intramolecular Diels−Alder cycloaddition recorded here again demonstrates an unexpected proclivity for the formation of the VM55599 relative stereochemistry which runs in sharp contradistinction to the stereochemical preference expressed in Nature. The steric effect of the methyl group in the β-methylproline ring is apparently not as important as originally thought in biasing the facial approach of the dienophile. Although the oxidation state of the putative azadiene species (**A**/**B**, X = O or H_2) in the biological system remains uncertain at this time, the contrast between the two biomimetic laboratory cycloaddition reactions[8c] and that postulated to occur in the biosynthetic constructions strongly implicates protein organization of the precyclization substrate conformation to greatly favor production of the paraherquamide relative stereochemistry. Whether protein *catalysis* of this construction occurs, remains an open question. Efforts to address these remaining subtle, yet significant, issues are the subject of ongoing studies in these laboratories.

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Supporting Information Available: Complete experimental procedures, spectroscopic and analytical data for all new compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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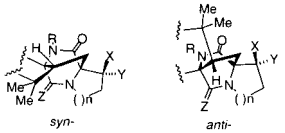
(16) The *syn*-/*anti*-relationship refers to the relative stereochemistry between the C-20 stereogenic center (VM55599 numbering) and the cyclic amino acid residue (proline, β-methylproline, or pipecolic acid):



The *syn*-selective cycloaddition referred to in Scheme 2 would result in diastereomers with the *syn* relative configuration as shown by the general structure above.

(17) The relative amount of paraherquamide to VM55599 harvested from *Penicillium* sp. IMI332995 is >600:1. This ratio does not include other paraherquamides (**1**−**12**) that are typically co-isolated with **1**.