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Paraherquamides, Brevianamides, and Asperparalines: Laboratory Synthesis and Biosynthesis. An Interim Report

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ABSTRACT

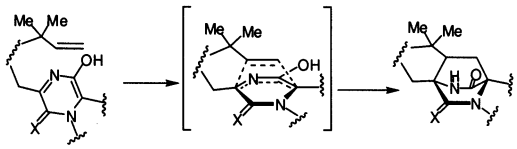
Studies from our laboratories on the paraherquamide, brevianamide, and asperparaline families of natural products are reviewed. It has been proposed that the unique core ring system that is common to this family of compounds arises by a biological intramolecular Diels−Alder cycloaddition reaction. Key biosynthetic studies are described, along with classical synthetic approaches as well as those inspired by Nature for the synthesis of these interesting molecules.

Introduction

The paraherquamides, brevianamides, and asperparalines constitute an unusual family of fungal metabolites that possess a unique bicyclo[2.2.2]diazaoctane core ring system that has been proposed to arise in Nature via a biological Diels−Alder reaction (Scheme 1). Despite its extensive use in synthetic chemistry, there are few proven examples of Diels−Alder reactions in biological systems, and fewer still reports of enzyme-catalyzed Diels−Alder reactions.[1]

There is, as yet, no report of an isolated Diels−Alderase from the fungi that produce the natural products that are the subject of this Account, but if this family of alkaloids are indeed biosynthesized via a [4 + 2] cycloaddition, the producing organisms may contain a rare but extremely important example of this type of enzyme.

Scheme 1. Proposed Diels−Alder Reaction Forming the Core Structure of the Brevianamides, Paraherquamides, and Asperparalines



The brevianamides comprise a small but structurally interesting family of indole alkaloids constructed from tryptophan, proline, and one isoprene unit (Figure 1). Brevianamide A was originally isolated as the major fluorescent metabolite from *Penicillium brevicompactum* in 1969.[2] Brevianamides B−F were later isolated from the same fungus,[3,4] although brevianamides C and D were subsequently demonstrated to be photochemically derived artifacts of isolation.[4] The structure and absolute stereochemistry of brevianamide A were secured through X-ray crystallography of a semisynthetic derivative,[5] but the absolute stereochemistry of brevianamide B was not determined. Brevianamides A and D have been shown to possess modest insecticidal activity.[6]

The paraherquamides are closely related to the brevianamides but are structurally more complex, being comprised of two isoprene units, tryptophan and variously substituted proline derivatives (Figure 2). Many members of this family display potent anthelmintic and antinematodal activities and are under investigation for use in veterinary medicine to treat intestinal parasites.[7]

The parent and most potent member, paraherquamide A, was isolated from cultures of *Penicillium paraherquei* by Yamazaki and co-workers in 1980.[8] Since then, paraherquamides B−G,[9] VM55595, VM55596, and VM55597,[10] SB203105 and SB200437,[11] and sclerotiamide[12] have been isolated from various *Penicillium* and *Aspergillus* species. Several closely related compounds have also been reported: marcfortines A−C possess a pipecolic acid unit in place of proline,[13] and VM55599,[10] aspergamides A and B,[14] and CJ-17,665[15] contain a 2,3-disubstituted indole rather than a *spiro*-oxindole.

The asperparalines differ from the brevianamides and paraherquamides in that they contain a *spiro*-succinimide ring system in place of the *spiro*-oxindole (Figure 3). Asperparalines A−C were isolated from *Aspergillus japonicus* JV-23 and shown to have paralytic effects on silkworms.[16] Asperparaline A was also isolated alongside the paraherquamides SB203105 and SB200427, and from the same fungus was also obtained 16-keto aspergillamide.[11]

The first significant investigations into the biosynthesis of this family were carried out on brevianamide A by Birch and co-workers.[3] Feeding experiments with labeled compounds and co-occurrence of postulated biosynthetic precursors with similar natural products[17] allowed them

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Rhona J. Cox was born in 1975 in Newcastle-upon-Tyne, England. She received her B.A. in chemistry in 1997 from the University of Oxford and continued there to undertake doctoral research under the supervision of Professor Sir Jack E. Baldwin. After the completion of her D.Phil. in 2001, she moved to Colorado State University to commence postdoctoral research in the group of Professor Robert M. Williams. Her research interests include the biosynthesis and biomimetic synthesis of natural products.

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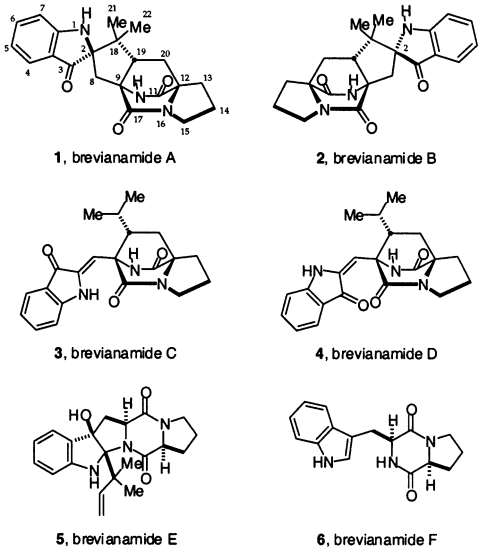


FIGURE 1. Structures of the brevianamides.

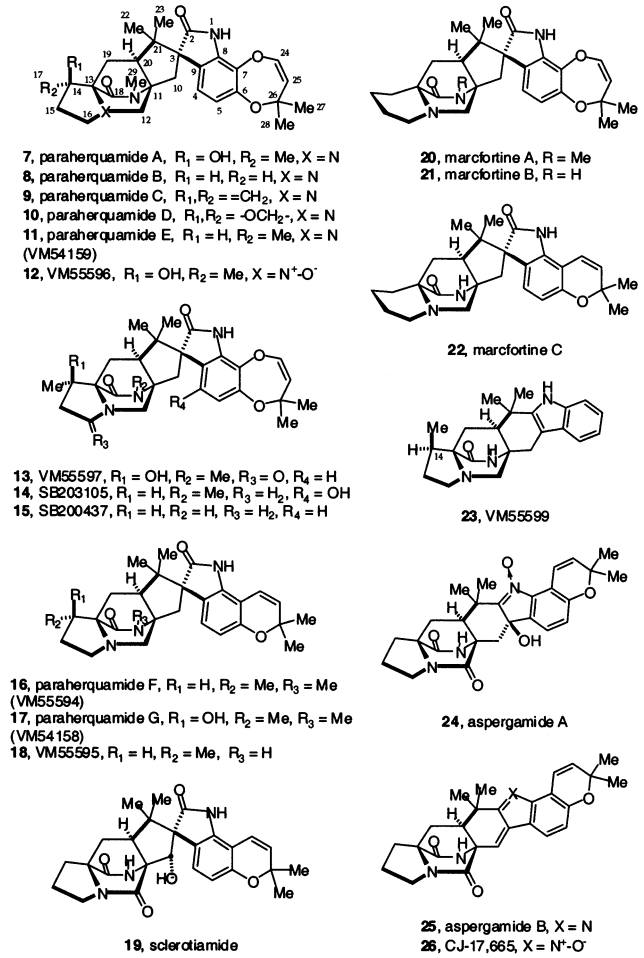


FIGURE 2. Structures of the paraherquamides and related alkaloids.

to propose the biosynthesis from tryptophan and proline, as depicted in Scheme 2.[18]

The introduction of the C_5 isoprene unit and the mechanism of formation of the novel bicyclo[2.2.2]-diazaoctane core ring system have been subjects of much speculation. Porter and Sammes were the first to suggest the involvement of an intramolecular Diels–Alder reaction

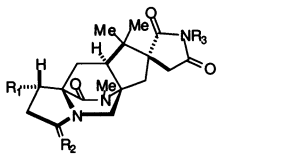
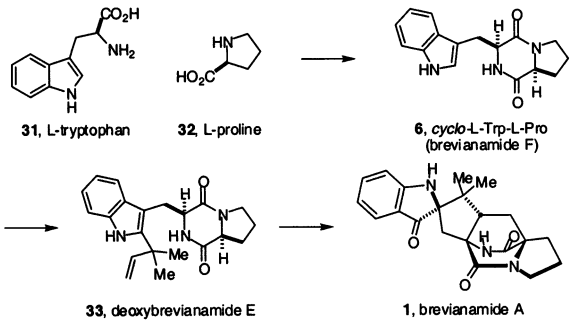
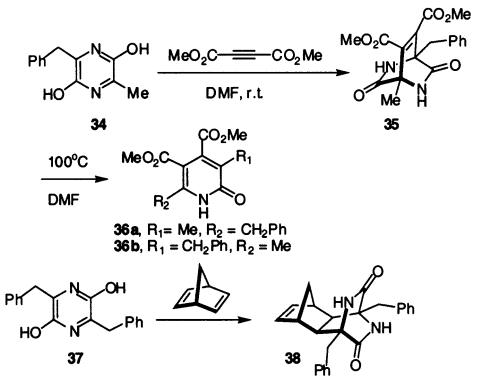


FIGURE 3. Structures of the asperparalines.

Scheme 2. Postulated Biosynthesis of Brevianamide A Proposed by Birch et al.[18]



Scheme 3. Biomimetic Diels–Alder Cycloadditions[19]

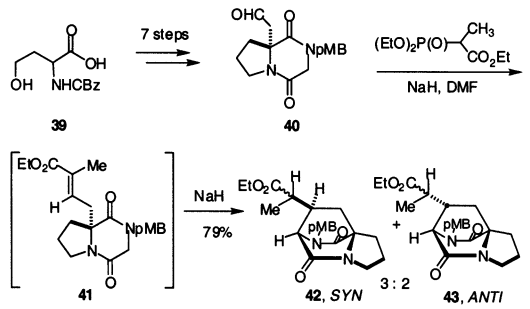


for the formation of this ring system, and the chemical feasibility of this type of reaction was demonstrated by a number of cycloadditions on pyrazine derivatives[19] (Scheme 3), but no further work on these compounds appeared in the literature for more than a decade. More recent biosynthetic investigations will be presented and discussed following the section on synthetic studies.

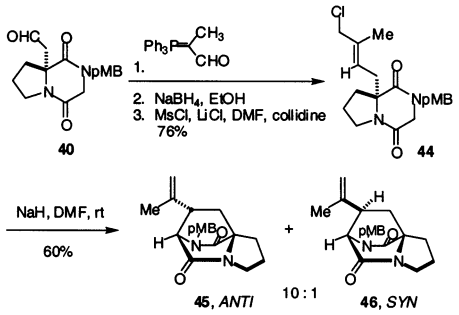
Synthetic Studies on the Brevianamides

In 1986, our group reported two cyclization reactions to form the bicyclo[2.2.2]diazaoctane ring system.[20] The substituted diketopiperazine **40** was constructed in seven steps from (±)-*N*-carbobenzyloxy homoserine **39**. A Horner–Wadsworth–Emmons olefination procedure provided the unsaturated ester **41**, which underwent immediate enolate formation and an intramolecular Michael-type cyclization to give the desired tricyclic products as a 3:2 mixture of two diastereomeric pairs, favoring the undesired syn relative configuration of the newly created stereogenic center (Scheme 4).

Scheme 4. Initial Michael Cyclization Model Studies on the Brevianamides[20]



Scheme 5. Initial S_N2' Cyclization Model Studies on the Brevianamides[20]

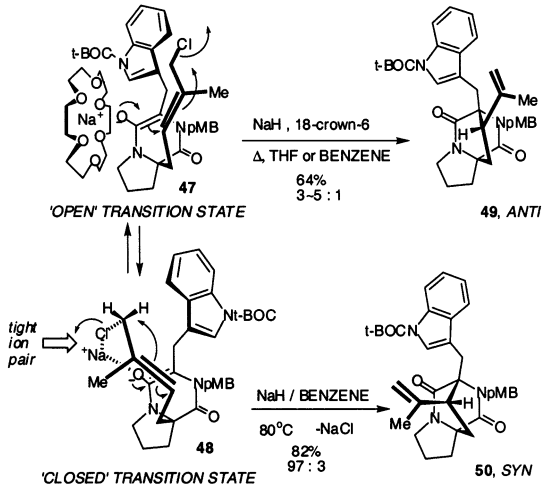


An alternative approach explored the utility of an intramolecular S_N2' cyclization. Wittig homologation of aldehyde **40**, followed by reduction and chlorination, gave the *E*-allylic chloride (**44**). Subjecting this species to NaH in *N*,*N*-dimethylformamide (DMF) resulted in the formation of two tricyclic products in 60% yield and in a 10:1 ratio, favoring the desired (brevianamide) anti relative stereochemistry (Scheme 5).

A number of substrates were examined and the metal counterion, solvent, temperature, and additives varied to observe the effect on the facial selectivity of the S_N2' reaction.[21] It was found that, in the presence of a polar solvent such as DMF or a metal-complexing ligand such as 18-crown-6, the anti product predominated, whereas in a nonpolar solvent such as benzene, the diastereoselectivity was completely reversed to favor the syn relative stereochemistry.

These results were rationalized in terms of "open" and "closed" transition states, as illustrated in Scheme 6. In the presence of a strongly coordinating species, a solvent ligation sphere surrounds the enolate metal counterion and sterically forces the allylic chloride to adopt an "open" transition state, whereas in a nonpolar solvent, the metal counterion and chloride leaving group form a tight, intramolecular contact ion-pair and reaction takes place through the "closed" transition state. However, better selectivity for the anti diastereomer is obtained when the metal ion and ligand are "mismatched", e.g., Na with 18-crown-6. When the pair is "matched", e.g., Na with 15-crown-5, the metal ion is coordinated so strongly that it is separated from the enolate faster than S_N2' cyclization can take place, and the reaction exhibits poor facial selectivity.

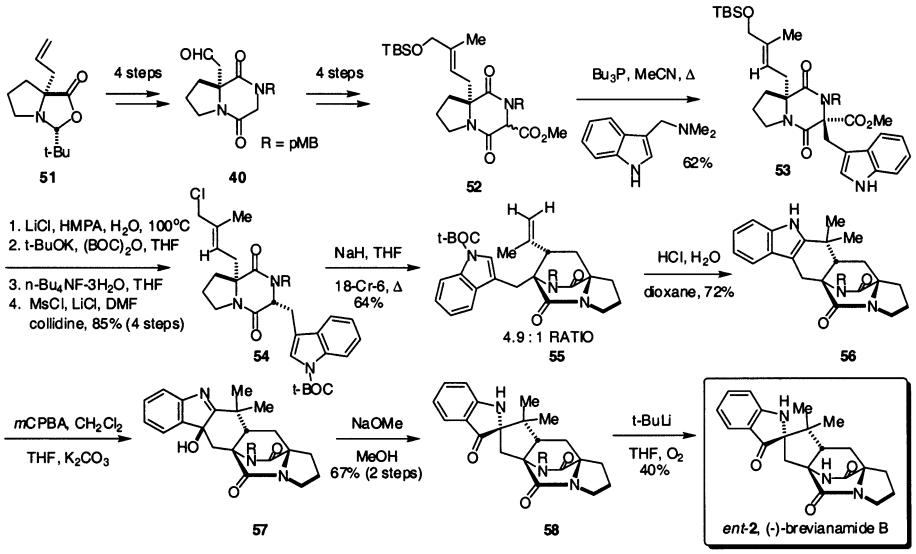
Scheme 6. Transition-State Models for the S_N2' Cyclization[21]



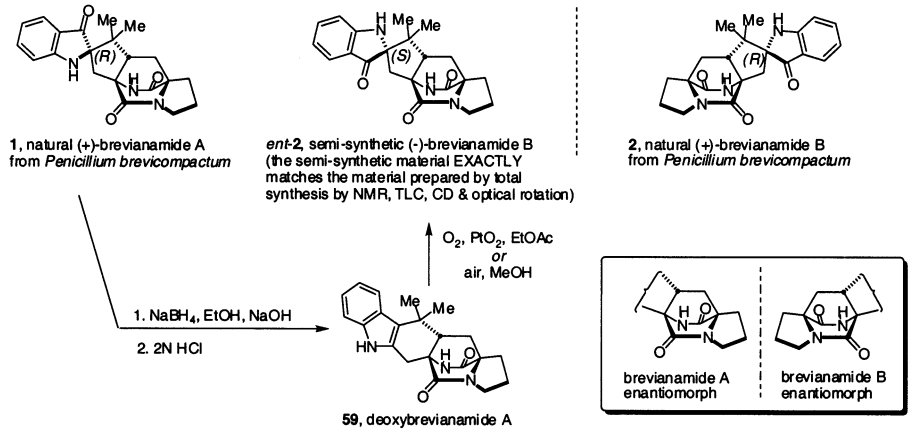
The intramolecular S_N2' cyclization was exploited in the asymmetric total synthesis of (−)-brevianamide B, as illustrated in Scheme 7.[21] The aldehyde **40** was prepared in enantiomerically pure form from known heterocycle **51**. Elaboration of the diketopiperazine and incorporation of the indolyl side chain were followed by the key intramolecular S_N2' cyclization reaction to furnish the bicyclo[2.2.2]diazaoctane nucleus. The diastereoselectivity of this reaction was between 3:1 and 5:1, favoring the desired anti configuration. Olefin-cation cyclization with concomitant removal of the butyloxycarbonyl (Boc) protecting group afforded hexacyclic compound **56**. Stereoselective oxidation of the 2,3-disubstituted indole was followed by a pinacol-type rearrangement to provide *spiro*-indoxyl **58**. Removal of the *N*-*p*-methoxybenzyl protecting group under standard conditions was unsuccessful, but after extensive experimentation it was found that treatment of **58** with excess *tert*-butyllithium in tetrahydrofuran at low temperature, followed by quenching with O_2, afforded (−)-brevianamide B in 40% yield. In connection with this work, we reported the removal of the *N*-*p*-methoxybenzyl group, by benzylic carbanion formation and oxidation, from a series of compounds generated from this work.[26] While the yields are modest (21−40%), this method offers an alternative to the oxidative conditions that are typically used to remove *N*-*p*-methoxybenzyl residues, such as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) or ceric ammonium nitrate (CAN).

The brevianamide B obtained by total synthesis was identical by ^{1}H NMR, IR, TLC, and UV to an authentic sample of brevianamide B obtained from fungal cultures. However, the specific rotations of the natural and synthetic samples were of opposite sign and of equal magnitude, indicating that the synthetic material is the enantiomorph of the natural product. Conversion of natural (+)-brevianamide A to semisynthetic brevianamide B by a method originally described by Birch[4] produced the (−)-enantiomer (Scheme 8), which demonstrates that brevianamides A and B are enantiomorphic with respect to the bicyclo[2.2.2] moiety but that each

Scheme 7. Asymmetric Synthesis of (−)-Brevianamide[21]



Scheme 8. Stereochemical Relationships within the Brevianamides[21]



metabolite has the (*R*) absolute configuration at the *spiro*-ψ-indoxyl stereogenic center.

Biosynthetic Studies on the Brevianamides

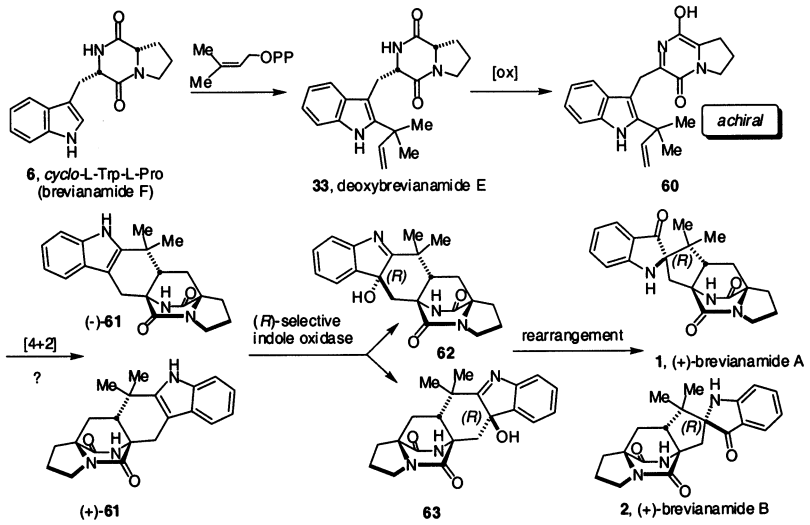
On the basis of the unexpected stereochemical relationship between brevianamides A and B, a biogenetic pathway was proposed that could accommodate the formation of the two enantiomorphic bicyclo[2.2.2] ring systems (Scheme 9).[22] Reverse prenylation of brevianamide F (**6**) to deoxybrevianamide E (**33**), as proposed by Birch, would be followed by oxidation and enolization to provide an achiral azadiene. Cycloaddition could, in principle, occur from either face of the azadiene system to yield the racemic (or partially racemic) hexacyclic indole cycloaddition products (**61**). Since both natural brevianamides A and B have the (*R*) absolute stereochemistry at the indoxyl spiro center, the involvement of an (*R*)-selective indole oxidase was invoked to effect the "resolution" of the hypothetical racemic Diels−Alder adduct into two optically pure, diastereomeric hydroxyindolenines. Stereospecific pinacol-type ring contraction would provide the optically pure brevianamides.

To test this hypothesis, we synthesized, in racemic form, the hypothetical Diels−Alder cycloadduct (**61**) bearing a ^{13}C label.[23] Feeding of this potential precursor to cultures of *Penicillium brevicompactum* failed to yield labeled brevianamides A and B (Scheme 10). In addition, evidence for the presence of unlabeled **61** could not be found in the fungus.

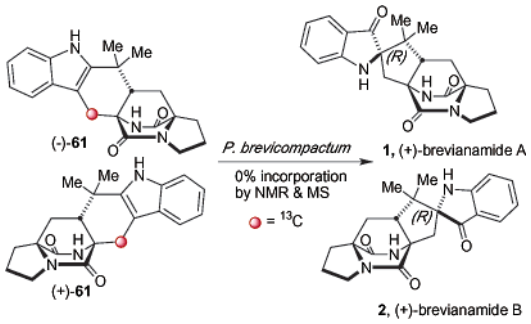
These results do not exclude the possibility that the hexacyclic indole is a biosynthetic precursor to the brevianamides because, for example, poor solubility or cell penetration may limit the access of the labeled, synthetic precursor to the biosynthetic machinery in the cells. However, enough doubt had been cast on the pathway depicted in Scheme 9 that we pursued alternatives.

The synthesis of tritium-labeled deoxybrevianamide E was carried out to probe the intermediacy of this compound in the biosynthesis of brevianamides A, B, and E.[23] It was found that this substance, when fed to cultures of *P. brevicompactum*, was efficiently incorporated into brevianamides A, B, and E. When radioactive brevianamide E was re-fed to the cultures, there was no significant incorporation into either brevianamide A or B, suggesting that brevianamide E is a dead-end, shunt metabolite. On

Scheme 9. Early Proposal for Brevianamide Biosynthesis[22]



Scheme 10. Biosynthetic Feeding of the Hexacyclic Indole[23]



the basis of the available data, we proposed an alternative biosynthetic pathway (Scheme 11). Reverse prenylation of brevianamide F to deoxybrevianamide E, followed by an (*R*)-selective oxidation at the 3-position of the indole, gives an (*R*)-hydroxyindolenine (**64**) which undergoes either irreversible nucleophilic addition of the tryptophyl amide nitrogen, forming brevianamide E, or a stereospecific pinacol-type rearrangement to a ψ-indoxyl (**65**). This substance would undergo two-electron oxidation of the tryptophyl α-carbon, maybe through a hydroxamic acid intermediate, then enolization and finally intramolecular Diels–Alder cycloaddition, possibly catalyzed by metal coordination to the azadiene, from two of the four possible diastereomeric transition states to yield brevianamides A and B.

Ab initio calculations were carried out on the four possible transition structures for the Diels–Alder reaction of **67**, as illustrated in Scheme 12.[24] It was found that the lowest energy transition-state structure, **TSA**, is that which would give rise to brevianamide A, the major metabolite. The next highest energy transition state, **TSB**, would lead to brevianamide B, obtained from *P. brevicompactum* in much smaller amounts. The C-19-*epi* products arising from the two highest energy transition-state structures have not been detected in the producing fungus.[21]

Extensive efforts in our laboratories to prepare key intermediate **65** have been unsuccessful, and it appears that this substance is unstable, decomposing via a retro Michael reaction. In addition, the isolation of VM55599 lends weight to our earlier hypothesis that the Diels–Alder cyclization occurs prior to oxidation and ψ-indoxyl formation. Further work is needed to explore the timings of cycloaddition, oxidation, and pinacol-type rearrangement.

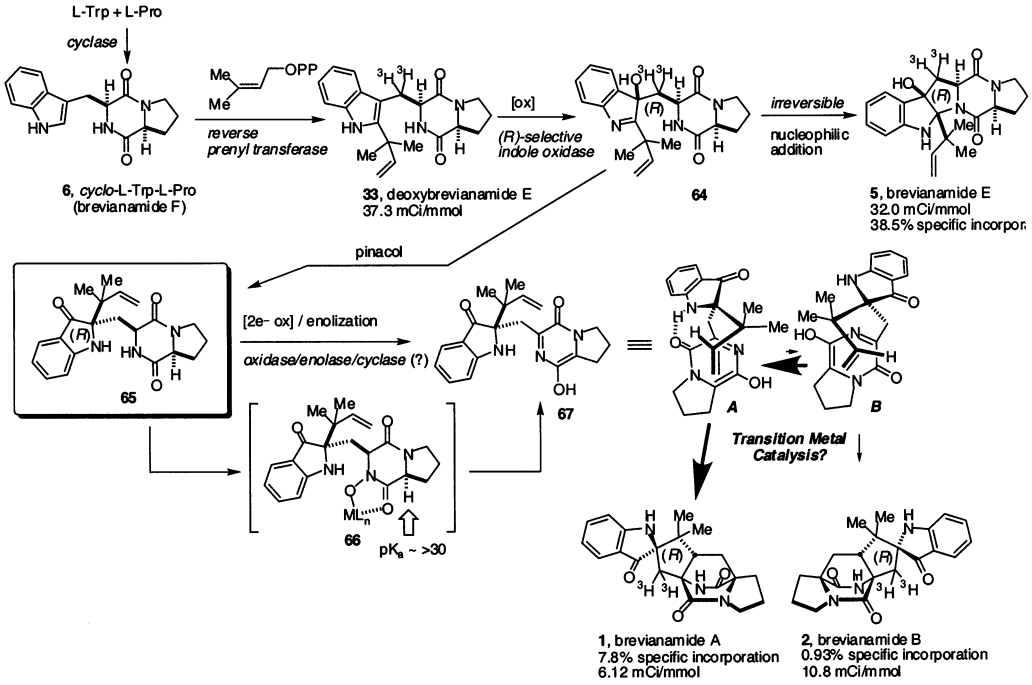
A Biomimetic Approach to the Brevianamides

Synthesis of the brevianamides using a Diels–Alder reaction is an attractive route to these compounds, and in 1998 we reported the first successful potentially biomimetic route to racemic brevianamide B (Scheme 13).[25] 9-*Epi*-deoxybrevianamide E (**70**) was converted to the corresponding lactim ether. Oxidation and isomerization to the labile azadiene were followed by spontaneous cyclization to yield a mixture of **74** and **75**, the ratio of products being largely unaffected by the solvent. Conversion of the minor isomer (**75**) to brevianamide B was accomplished in four steps. In a similar fashion, the major isomer from the Diels–Alder reaction was converted to C-19-*epi*-brevianamide A, a non-natural isomer of brevianamide A previously synthesized in this laboratory.[26]

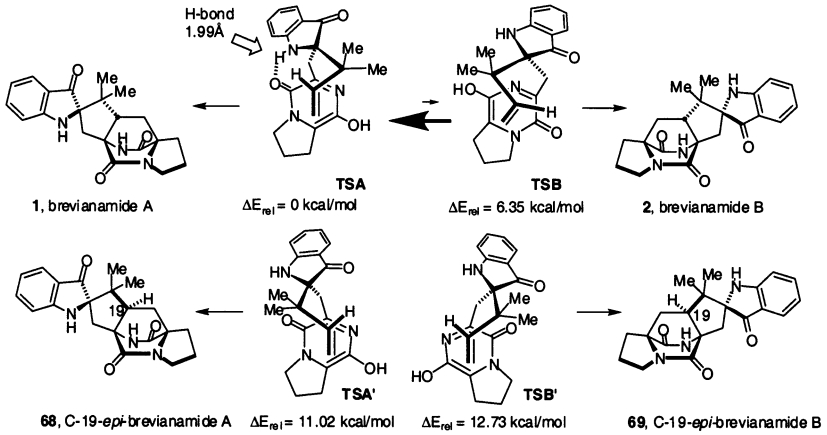
This study demonstrates that formation by an intramolecular Diels–Alder cyclization of the core bicyclo[2.2.2]-diazaoctane ring system common to this family of natural products is chemically feasible. However, since there are no reports of C-19-*epi*-metabolites from *P. brevicompactum* or of similarly epimeric compounds from the organisms that produce the paraherquamides or asperparalines, it would appear that, unlike in the laboratory synthesis, in the biosynthetic case there must be complete facial selectivity in the construction of the bicyclo[2.2.2] ring nucleus, lending weight to the proposal that an enzyme is involved in the Diels–Alder reaction.

We also examined some model intramolecular Diels–Alder reactions.[27] Azadiene **82**, formed by tautomerization of **81** under basic conditions, underwent immediate cycloaddition to give the tetracyclic products with anti diastereoselectivity, and no trace of the syn diastereomer could be found (Scheme 14).

Scheme 11. Later Proposal for Brevianamide Biosynthesis[23]



Scheme 12. Ab Initio Calculations on the Putative Diels–Alder Cycloadditions[24]



Curiously, in a related study by Liebscher and coworkers,[28] treatment of (*Z*)-ylidene piperazinedione **84** with acetyl chloride provided a bicyclo[2.2.2]diazaoctane system with complete syn stereoselectivity, corresponding to the stereochemistry observed in the paraherquamides rather than that observed in the brevianamides (Scheme 15). The reasons for the difference in selectivity between this reaction and the Diels–Alder reaction illustrated for the synthesis of brevianamide B (Scheme 13) are at present uncertain.

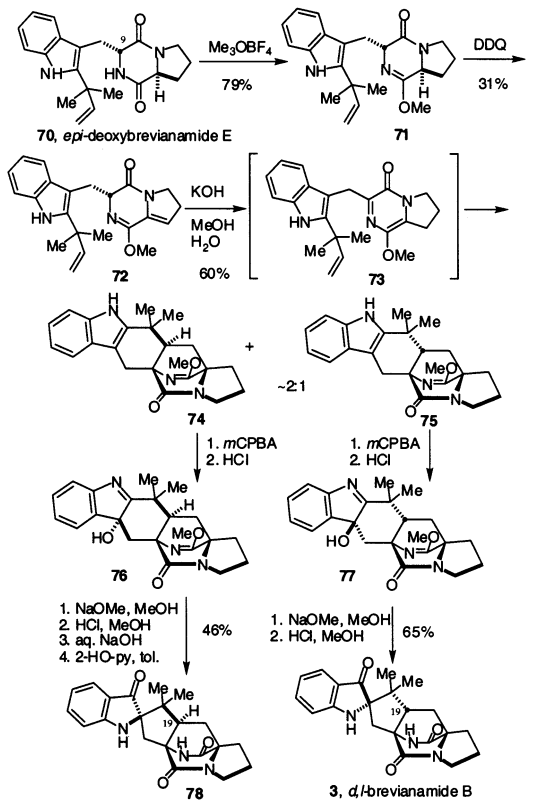
Synthetic Studies on Paraherquamides A and B

Unlike the brevianamides, the paraherquamides possess syn stereochemistry about the bicyclo[2.2.2]diazaoctane core and contain a *spiro*-oxindole rather than a *spiro*-indoxyl. In addition, the oxindole portion of the molecule is fused to a six- or seven-membered ring, and the proline unit is in some cases more heavily substituted.

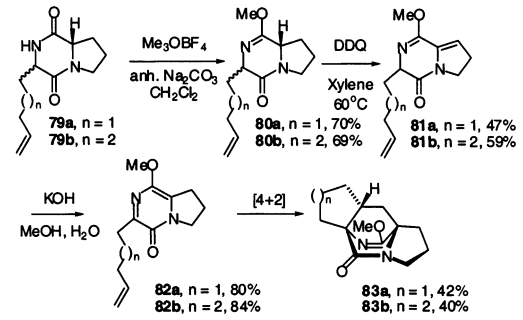
Our first synthetic model study on the paraherquamides utilized a hexacyclic indole with syn stereochemistry (**86**), generated during the course of our work on brevianamide B.[29] Treatment of this substance with *tert*-butyl hypochlorite followed by aqueous acetic acid generated not the *spiro*-indoxyl, as is required for the brevianamide core structure, but a mixture of diastereomeric *spiro*-oxindoles, with the major isomer (**89**) possessing the paraherquamide relative stereochemistry (Scheme 16).

Thus, we envisioned that the paraherquamides could now be synthesized using a route similar to our first synthesis of brevianamide, altering the conditions for the key S_N2' cyclization to allow for predominant formation of the stereoisomer with syn relative stereochemistry. New methodology was developed to allow incorporation of the 2*H*-1,5-benzodioxepin ring system found in both metabolites[30] (Scheme 17) and the β-hydroxy-β-methylproline residue found in paraherquamide A.[31] Syntheses of the

Scheme 13. Biomimetic Total Synthesis of Racemic Brevianamide B[25]



Scheme 14. Model Intramolecular Diels–Alder Cycloadditions[27]



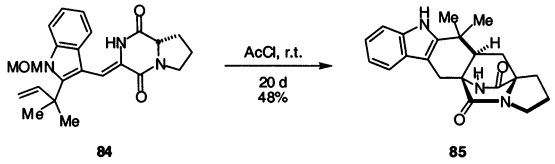
unnatural isomer of paraherquamide B[32] and the natural isomer of paraherquamide A[33] are illustrated in Schemes 18 and 19.

Biosynthetic Studies on the Paraherquamides

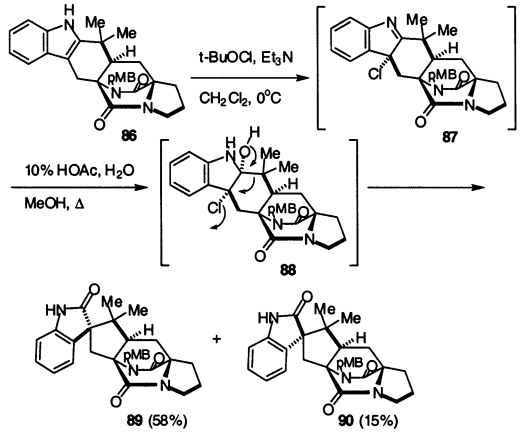
We have recently investigated the origin of the β-methylproline moiety present in paraherquamide A and several congeners. Through feeding of labeled amino acids to the producing organism, the β-methylproline unit was found to be derived not from methylation of proline, but from L-isoleucine. Feeding studies with dipeptides composed of tryptophan and isoleucine were inconclusive, and it seems more likely that isoleucine is converted to β-methylproline before conjugation to tryptophan, although other possibilities exist.[34]

Oxidative cyclization of the nitrogen atom onto the C-5 methyl group in isoleucine is an unusual biosynthetic transformation. Two reasonable pathways are depicted in

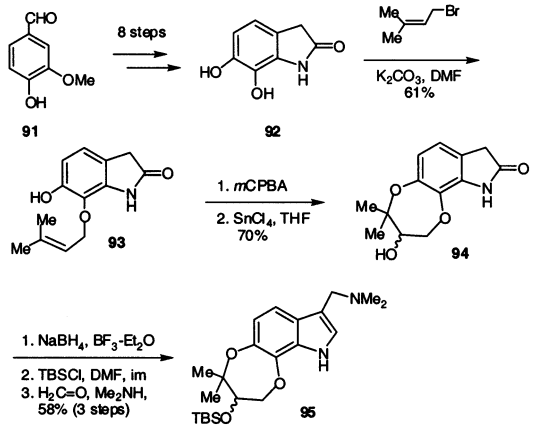
Scheme 15. Diels-Alder Cycloaddition Reported by Liebscher et al.[28]



Scheme 16. Oxindole Model Study[29]



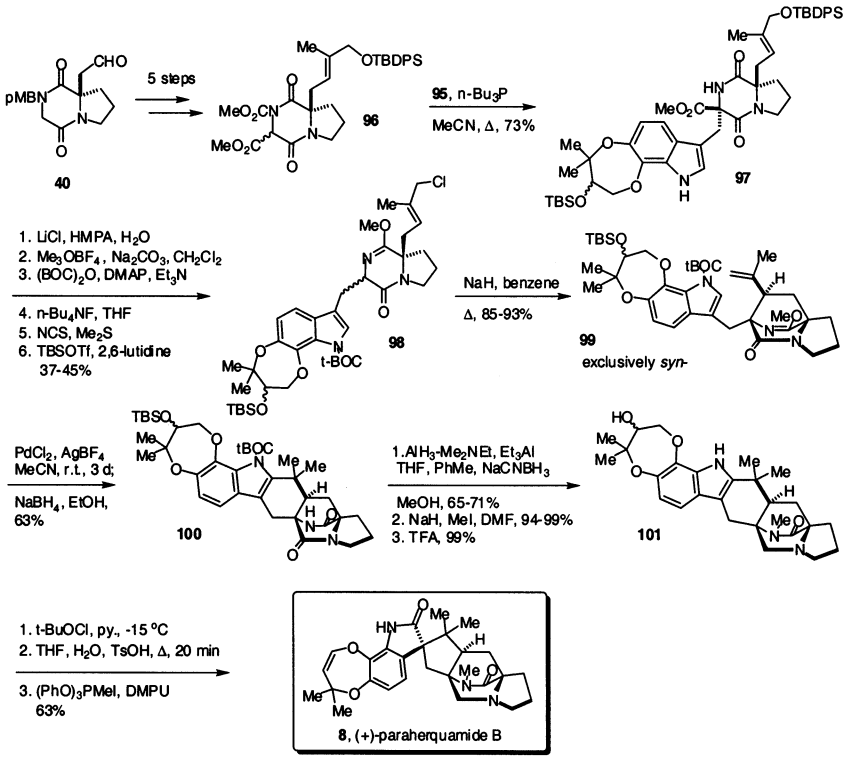
Scheme 17. Construction of the Dioxepin Gramine Derivative[30]



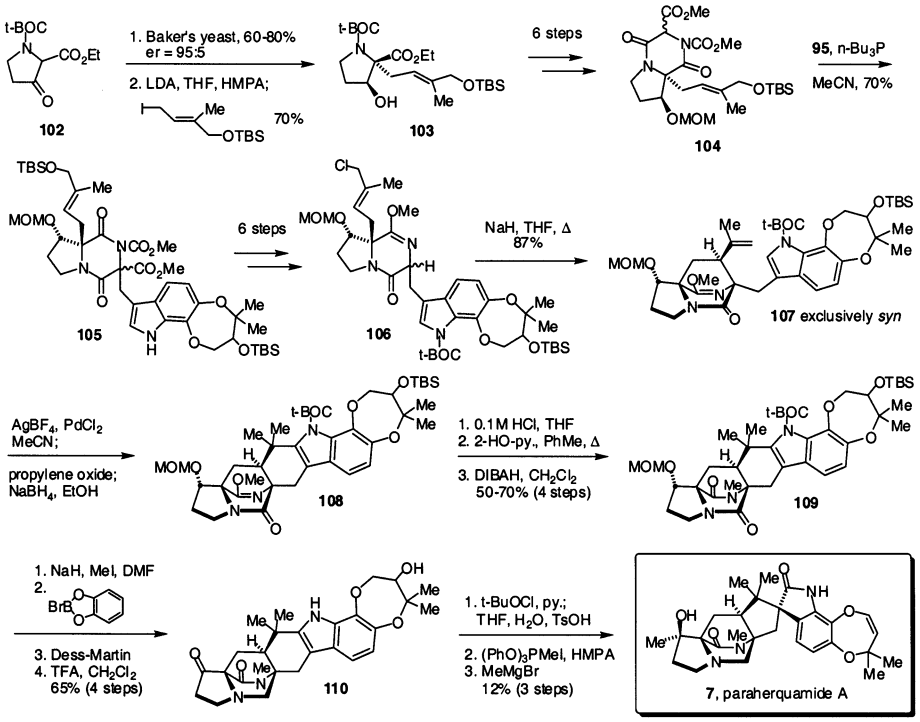
Scheme 20. One involves four-electron oxidation of the distal side-chain methyl group to an aldehyde, followed by cyclization and loss of water to produce an iminium species; subsequent reduction furnishes the β-methylproline derivative. Alternatively, direct functionalization of the C-5 methyl group by, for example, chlorination, and displacement can also be envisioned to lead to β-methylproline. We distinguished these two possible mechanisms by a feeding study with deuterium-labeled isoleucine.[35] The paraherquamide A produced contained a β-methylproline residue which contained only one deuterium atom, indicating that cyclization of L-isoleucine occurs, in this case, through an initial four-electron oxidation.

In the course of isotopic labeling studies aimed at examining the origin of the C_5 isoprene units in the paraherquamide structure, we discovered an unexpected stereochemical distribution of the geminal methyl groups derived from dimethylallyl pyrophosphate (DMAPP).[36] Specific incorporation of intact C_2 units from ^{13}C-labeled acetate was observed, which suggests that the isoprene

Scheme 18. Total Asymmetric Synthesis of (+)-Paraherquamide B[32]



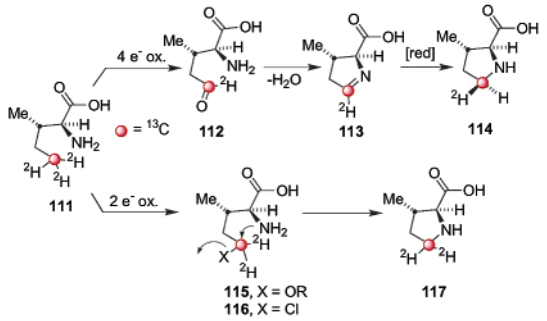
Scheme 19. Total Asymmetric Synthesis of (−)-Paraherquamide A[33]



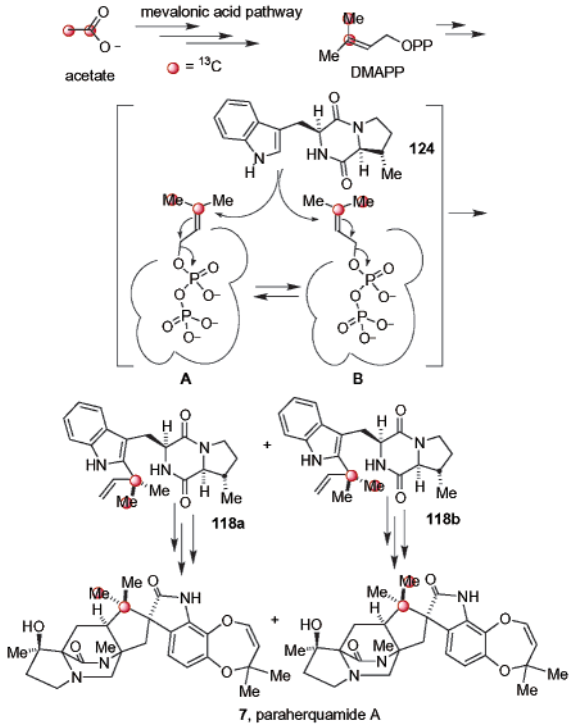
units arise from the classical mevalonate pathway. However, in the isoprene unit which forms part of the bicyclo[2.2.2] core, both geminal methyl groups show coupling to the quaternary carbon center, although not simultaneously. This suggests that at some point in the biosynthesis these two methyl groups become essentially equivalent. In contrast, in the isoprene fragment in the dioxepin ring, only one of the methyl groups shows coupling to the quaternary center, implying that the stereochemical integrity of DMAPP in forming this ring system is left intact.

This unexpected result was interpreted to mean that a reverse prenyl transferase presents the olefinic π-system of DMAPP in a manner in which both faces of the π-system are susceptible to attack by the indole at the 2-position. The simplest explanation is to invoke binding

Scheme 20. Mechanism of the Oxidative Cyclization of L-Isoleucine to β-Methylproline[35]



Scheme 21. Proposal for Incorporation of the Reverse Prenyl Group[36]



of DMAPP in an "upside-down" orientation (relative to "normal" prenyl transferases), which permits a facially nonselective S_N' attack on the π-system, as shown in Scheme 21. We speculate that in this situation the pyrophosphate group is anchored in the enzyme active site, with the hydrophobic isopropenyl moiety being presented in a conformationally flexible disposition with respect to the tryptophan-derived substrate. In contrast, the normal mode of prenyl transfer involves nucleophilic displacement at the pyrophosphate-bearing methylene carbon, with the hydrophobic tail of DMAPP buried in the enzyme active site. It seems likely that a normal prenyl transferase is used to introduce the isoprene unit in the dioxepin ring, with direct alkylation being followed by a stereospecific net oxidative addition to the olefinic system. This work demonstrates the first case where both a non-face-selective and a face-selective addition to the trisubstituted olefinic portion of DMAPP has occurred within the same molecule. Much work remains to more fully establish the sequence of events involved in the biosynthesis of the paraherquamides.

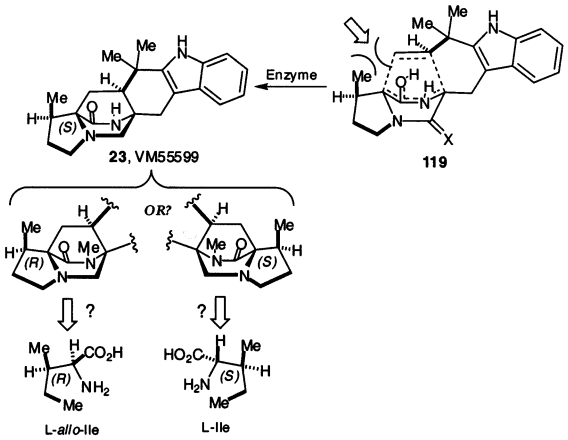


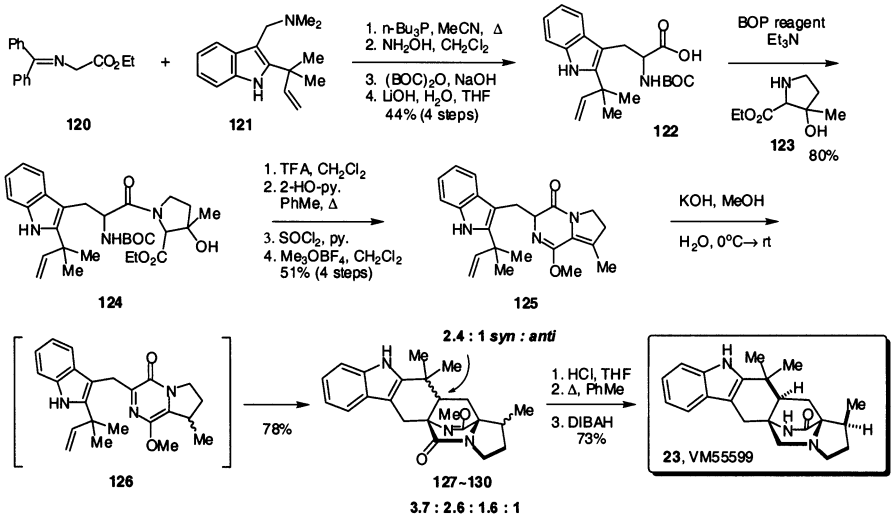
FIGURE 4. The VM55599 stereochemical paradox.[34]

Biomimetic Total Synthesis of VM55599

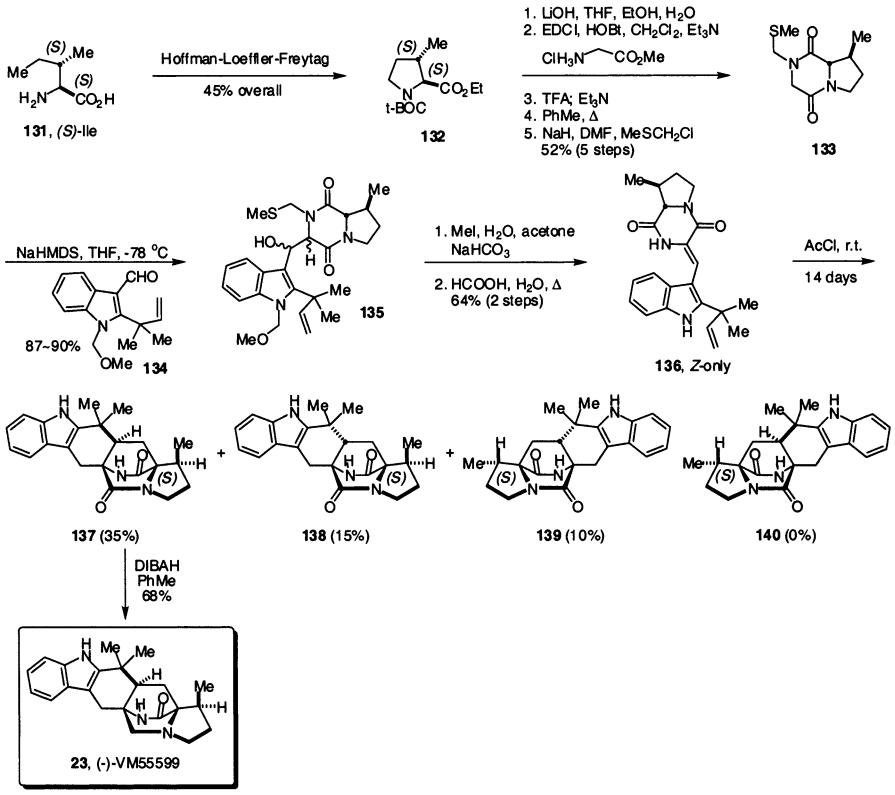
The identification of L-isoleucine as the biosynthetic building block of paraherquamide A has posed an interesting stereochemical paradox with respect to the origin and stereochemistry of β-methylproline in the natural metabolite VM55599, isolated from a paraherquamide-producing fungus.[10] The relative (but not absolute) stereochemistry was determined by the group who originally isolated the compound.[10] Significantly, the methyl group in the β-methylproline ring of VM55599 is disposed syn to the bridging isoprene moiety, whereas in all the other paraherquamides and asperparalines isolated to date, the anti stereochemistry is observed.

Assuming that the absolute stereochemistry of the bicyclo[2.2.2]diazaoctane core of VM55599 is the same as that found in the paraherquamides, the stereochemistry at C-14 was assigned as (*R*), which is the *opposite* to that found in paraherquamides.[33] The side-chain stereochemistry of L-isoleucine is preserved in the biosynthesis of paraherquamide A, with hydroxylation at C-14 proceeding with net retention of configuration, so if L-isoleucine is also the precursor to the β-methylproline ring of VM55599, it must follow that the bicyclo[2.2.2]diazaoctane ring system of this compound must be *enantiomorphic* to that of the paraherquamides. Alternatively, VM55599 may be derived from L-*allo*-isoleucine; this would result in the (*R*) stereochemistry at C-14 and would accommodate the same bicyclo[2.2.2]diazaoctane ring system absolute stereochemistry as paraherquamide A. Finally, since the methyl group of the β-methylproline ring of VM55599 is syn to the isoprene unit comprising the bicyclo[2.2.2]-diazaoctane ring system, this implies that the putative biosynthetic Diels–Alder cyclization to form this system occurs from the more hindered face of the azadiene system (Figure 4).[34] The stereochemical paradox posed by VM55599 raises interesting questions concerning the biogenesis of these substances: Is VM55599 a biosynthetic precursor to various paraherquamides? Or, is VM55599 a minor shunt metabolite with the opposite absolute stereochemistry of the bicyclo[2.2.2]diazaoctane

Scheme 22. Racemic Biomimetic Total Synthesis of VM55599[37]



Scheme 23. Asymmetric Synthesis of (−)-VM55599[38]

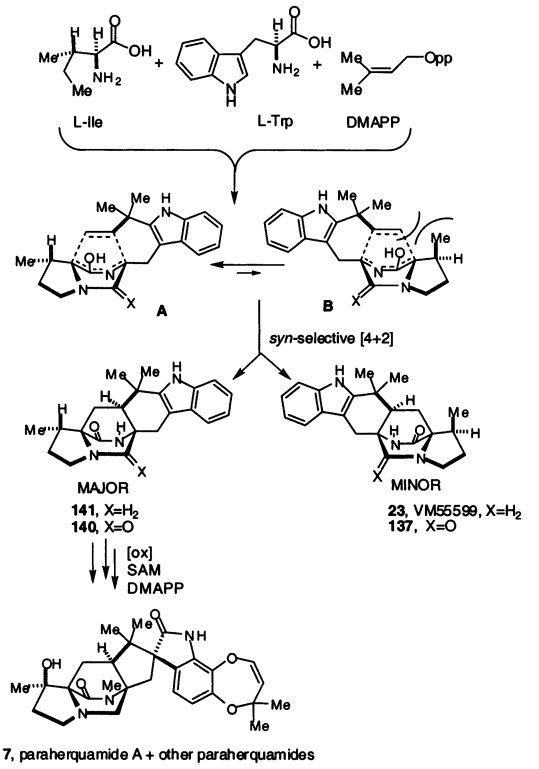


ring system? The mechanism of formation of the bicyclo-[2.2.2]diazaoctane ring system in both series posed an interesting stereochemical and enzymological puzzle that has been recently solved in our laboratories.

To ascertain the absolute stereochemistry of VM55599, a racemic synthesis was first carried out, as depicted in Scheme 22.[37] This synthetic route utilizes the same Diels–Alder reaction that was used in our biomimetic synthesis of brevianamide B to give a separable mixture of all four possible diastereomers. Unexpectedly, the major products (**127** and **129**) in each diastereomeric subset displayed the methyl group in the β-methylproline ring syn to the bridging isoprene unit, with a diastereoselectivity of 1.47:1. Although it is reasonable to expect only modest diastereoselectivity for this cycloaddition, the methyl group on the proline ring was expected to exert a slight steric bias toward cycloadducts in which the methyl group is anti to the bridging isoprene moiety. Cycloadduct **127** was converted to racemic VM55599, and the synthetic sample was used to guide re-isolation of 0.4 mg of naturally occurring VM55599 from 12 L of solid media.

An asymmetric synthesis of VM55599 was next undertaken to establish the absolute configuration of this metabolite (Scheme 23).[38] The stereochemistry of β-methylproline is set at the start of the sequence by obtaining this material from L-isoleucine using a Hoffmann–Loef-

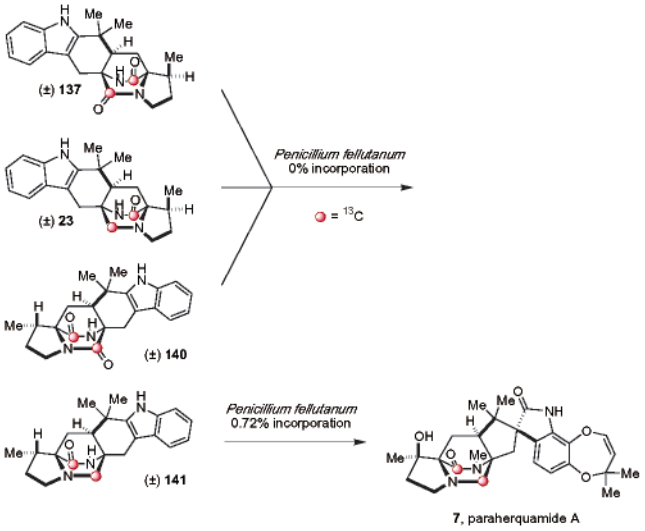
Scheme 24. Unified Biogenesis of the Paraherquamides and VM55599[38]



fler−Freytag reaction.[39] Reaction of **136** with acetyl chloride for 14 days leads to the formation of an azadiene, which undergoes an immediate Diels−Alder reaction to produce three out of the four possible cycloadducts. As with the racemic synthesis of VM55599, the cycloaddition reaction displays a preference for the formation of the VM55599 relative stereochemistry, but the proportions of the other three diastereomers differ. It was surprising to observe that cycloadduct **140**, which contains the relative and absolute stereochemistry of the paraherquamides, was not detected from the cycloaddition reaction. Finally, reduction of the cycloadduct **137** gave synthetic (−)-VM55599 that was identical in all respects to natural (−)-VM55599, including optical rotation, CD, and retention time on chiral HPLC. Thus, natural VM55599 has the absolute configuration depicted in Scheme 23.

This result rigorously confirms the predicted absolute stereochemistry of VM55599 and provides experimental support for the unified biogenesis we propose for this compound and the paraherquamides (Scheme 24). In our 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 24). The major product of the cycloaddition would be compound **140** or **141**, wherein the dienophile attacks the opposite face of the azadiene, anti to the methyl group, which would give rise to the various paraherquamides.

Scheme 25. Biosynthetic Studies Using Doubly ^{13}C-Labeled Substrates[40]



This hypothesis was recently experimentally tested through the feeding of racemic, doubly ^{13}C-labeled compounds **23**, **137**, **140**, and **141** to *Penicillium fellutanum* (Scheme 25).[40] Isolation of the paraherquamide A produced showed no significant incorporation of **23**, **137**, or **140**. However, **141** was incorporated intact into paraherquamide A.

The implications of these observations are considerable. The failed incorporation of diketopiperazine **140** raises interesting questions concerning the timing of the reduction of the tryptophan-derived carbonyl group. The incorporation of (±)-**141** in significant isotopic yield indicates that the formation of the bicyclo[2.2.2]diazaoctane occurs before oxidations of the indole to form the *spiro*-oxindole and catechol moieties. It therefore follows that the dioxepin prenylation and *N*-methylation reactions must also occur late in the pathway. In addition, these results provide circumstantial evidence that VM55599 is a minor shunt metabolite and not an intermediate in the biosynthesis of the paraherquamides. Additional work needs to be done to elucidate the exact sequence of biosynthetic reactions immediately preceding and following the formation of **141**.

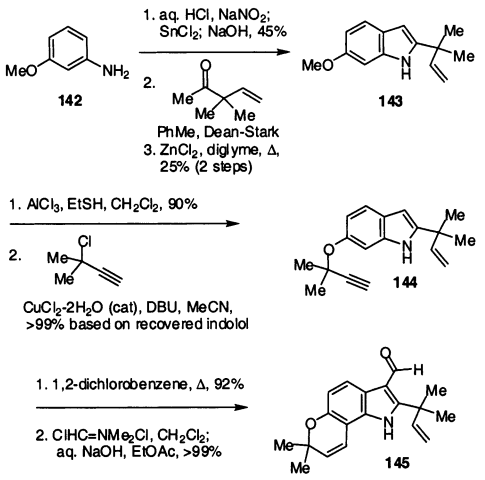
Synthetic Studies toward Paraherquamide F

The Diels−Alder reaction used to form the bicyclo[2.2.2]-diazaoctane core structure of VM55599 could potentially be used for the synthesis of other members of the paraherquamide family. We have recently reported the synthesis of a tricyclic compound (**145**) (Scheme 26) which, it is hoped, can be used in the synthesis of paraherquamide F and related compounds, following a synthetic strategy similar to that depicted in Scheme 23.[41]

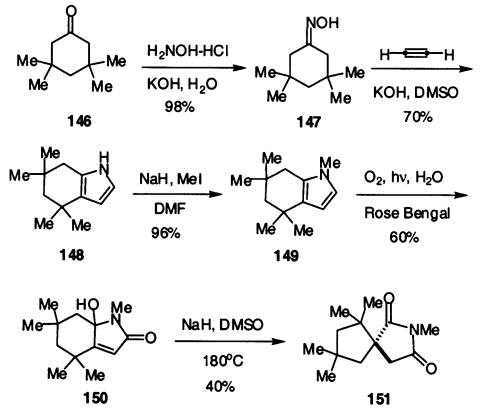
Synthetic Approach to Asperparaline

Isolation of the asperparalines was relatively recent, and thus far few details concerning their synthesis and biosynthesis have been elucidated either by our group or by

Scheme 26. Construction of a Dihydropyranoindole for the Synthesis of Paraherquamide F[41]



Scheme 27. Model Study on the *Spiro*-succinimide Ring System of Asperparaline A[42]



others working in the field. It seems reasonable to suppose that the *spiro*-succinimide of the asperparalines is biosynthesized by a route analogous to that proposed for the *spiro*-oxindoles in the paraherquamides. Our first synthetic model study toward asperparaline A utilizes oxidation of a pyrrole and subsequent rearrangement and is shown in Scheme 27.[42]

Initial results on the biosynthesis of the asperparalines have proved intriguing but will be published elsewhere.

Conclusion

The unusual structures and interesting biological activity of the paraherquamides, brevianamides, and asperparalines have made them important and challenging targets for synthetic and biosynthetic studies. During the course of our investigations on their synthesis and biosynthesis, many unexpected results and reactions have been discovered, and there is growing evidence for protein organization of the conformation of the putative substrates undergoing the biosynthetic intramolecular Diels–Alder cyclization reactions. However, much work is left to do before we have a full understanding of the biosyntheses of these substances, and work is ongoing in our laboratories to solve some of the mysteries that remain.

This work was supported by the National Institutes of Health. The authors are deeply indebted to our other co-workers who are cited in the references. Additional thanks to Dr. Jeremy Everett of Pfizer, Dr. Timothy Blizzard of Merck, and Dr. Byung H. Lee of Pharmacia for sharing preprints of their work and spectra of certain compounds that proved important for this work.

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