Studies on the Biosynthesis of Paraherquamide A. Origin of the β-Methylproline Ring

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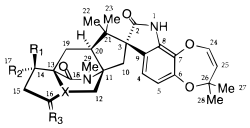
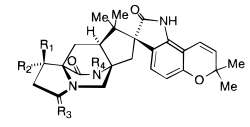
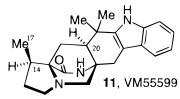
The paraherquamides are potent anthelmintic alkaloids isolated from various *Penicillium* sp.[1] These substances have attracted considerable attention due to their molecular complexity, intriguing biogenesis, and potential as antiparasitic drugs.[2] The most potent member of this family is paraherquamide A (**1**), which contains the unusual amino acid, β-methyl-β-hydroxyproline. The paraherquamides differ with respect to substitution and oxygenation in the proline ring and the prenylated oxindole ring; paraherquamide B (**2**) is the simplest member of the paraherquamide family, being comprised of the amino acids proline, tryptophan, and two isoprene units. Other members of this class include paraherquamides C-G, VM55596, VM55597, and VM55595. Recently, a Smith-Kline Beecham group reported the isolation and structural elucidation of the simpler indole alkaloid VM55599 (**11**) which, like compounds **5**−**10**, contains the unusual amino acid β-methylproline. As part of a program directed primarily at elucidating the biosynthetic mechanism of formation of the unique core bicyclo[2.2.2] ring system that is common to all of these alkaloids,[3] we have initiated studies on the biosynthesis of the unusual, functionalized proline derivatives that constitute the paraherquamide family. Herein, we report the biosynthetic incorporation of primary amino acid building blocks that constitute the core framework of this class of alkaloids.



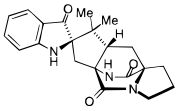
1, paraherquamide A, R_1 = OH, R_2 = Me, R_3 = H_2, X = N
2, paraherquamide B, R_1 = H, R_2 = H, R_3 = H_2, X = N
3, paraherquamide C, R_1 = R_2 = CH_2, R_3 = H_2, X = N
4, paraherquamide D, R_1 = O, R_2 = CH_2, R_3 = H_2, X = N
5, paraherquamide E, R_1 = H, R_2 = Me, R_3 = H_2, X = N
6, VM55596, R_1 = OH, R_2 = Me, R_3 = H_2, X = N$^+$-O$^-$
7, VM55597, R_1 = OH, R_2 = Me, R_3 = O, X = N



8, paraherquamide F, R_1 = H, R_2 = Me, R_3 = R_4 = Me
9, paraherquamide G, R_1 = OH, R_2 = Me, R_3 = R_4 = Me
10, VM55595, R_1 = H, R_2 = Me, R_3 = H_2, R_4 = H



11, VM55599



12, (+)-brevianamide A

Birch and associates carried out preliminary studies on the biosynthesis of the structurally related alkaloid brevianamide A (**12**), a substance that contains the core bicyclo[2.2.2] ring system.[4] These workers found that [15-^{3}H, 8-^{14}C]*cyclo*-L-tryptophan-L-proline (**13**), [3-^{14}C]-L-tryptophan, and [5-^{3}H]-L-proline were biosynthetically incorporated into brevianamide A in significant radiochemical yield. From these studies it seemed plausible that the monoketopiperazine ring system in paraherquamide A (**1**) might arise from tryptophan and proline. For paraherquamide A, C-14 methylation might arise via methyl transfer from *S*-adenosylmethionine to a 2,3-dehydroproline derivative followed by reduction. Close examination of the absolute stereochemistry of paraherquamide A, which possesses the (*S*)-absolute stereochemistry at C-14, led us to speculate that the methylated proline may instead be derived from L-isoleucine, and this possibility was experimentally tested.

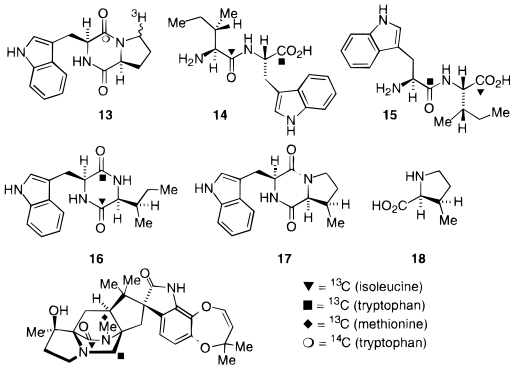


Figure 1.

To determine the primary metabolic building blocks that comprise the bicyclo[2.2.2] monoketopiperazine ring system of paraherquamide A, feeding experiments were performed on *Penicillium fellutanum* (ATCC: 20841) using [1-^{13}C]-L-tryptophan, [*methyl*-^{13}C]-L-methionine, and [1-^{13}C]-L-isoleucine (Figure 1). The position of ^{13}C incorporation in paraherquamide A was determined using ^{13}C NMR, and the percentage of the labeled amino acid incorporated was determined using ^{13}C NMR.[5] The [1-^{13}C]-L-tryptophan was incorporated, as expected (2.5%), with the label at C-12. The [methyl-^{13}C]methionine was not incorporated in the β-methylproline ring, but rather, only at C-29, the *N*-methyl position of the monoketopiperazine ring (0.6%). Feeding of [1-^{13}C]-L-isoleucine to *P. fellutanum* (ATCC: 20841), followed by harvesting the cells and isolation of paraherquamide A, revealed that the labeled L-isoleucine was incorporated into the monoketopiperazine ring system in high isotopic yield (3.3−3.7%) with the label at C-18. After determining the primary amino acid building blocks of the

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(5) For a leading reference on the method employed, see: Leete, E.; Bodem, G. B. *J. Am. Chem. Soc.* **1976**, *98*, 6321. These values were further corroborated by electrospray mass spectroscopy (see supporting information).

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paraherquamide A ring system, we attempted to further establish the structure of possible isoleucine/tryptophan conjugates on this pathway.

We have previously proposed a common biosynthetic route to the brevianamides and paraherquamides involving prenylation of *cyclo*-L-tryptophan-L-proline, oxidation of the indole followed by an oxidative [4 + 2] cycloaddition to provide the core bicyclo[2.2.2] ring system.[3] Since L-isoleucine forms the β-methylproline ring of paraherquamide A, *cyclo*-L-Trp-L-β-methylproline (**17**) or *cyclo*-L-Trp-L-Ile (**16**) are plausible precursors. There are numerous possible sequences of events that might occur in the formation of the final β-methylproline ring system. Formation of the dipeptides NH_2-L-Ile-L-Trp-COOH (**14**) or NH_2-L-Trp-L-Ile-COOH (**15**) and dehydration to *cyclo*-L-Trp-L-Ile (**16**) followed by oxidation of the terminal carbon of L-Ile and cyclization to form the β-methylproline moiety would result in *cyclo*-L-Trp-L-β-methylproline (**17**). Another possibility involves oxidation of the L-Ile followed by cyclization and reduction to afford the L-β-methylproline (**18**) followed by coupling to L-Trp to give *cyclo*-L-Trp-L-β-methylproline (**17**). Many other possibilities exist that would involve formation of the β-methylproline ring at a later stage.

We have investigated the simplest of these possibilities: doubly labeled NH_2-[1-^{13}C]-L-Ile-[1-^{13}C]-L-Trp-COOH (**14**); NH_2-[1-^{13}C]-L-Trp-[1-^{13}C]-L-Ile-COOH (**15**), and [2,5-$^{13}C_2$]-*cyclo*-L-Trp-L-Ile (**16**) were synthesized and fed to *P. fellutanum*. After the cells were grown and harvested as described above, 1.2−1.8% incorporation at C-18 and 0.4−0.9% incorporation at C-12 were evidenced by ^{13}C NMR. The ^{13}C NMR spectra of the paraherquamide A so produced did not provide compelling evidence for site-specific incorporation of both labels from the intact dipeptides.[5] This low level of incorporation is more consistent with dipeptide hydrolysis, reincorporation of the individual amino acids presumably coupled with additional metabolic degradation, and reconstitution of ^{13}C-enriched building blocks. Moreover, the mass spectra of the paraherquamide A isolated from these feeding experiments did not show the isotopic peak pattern expected from incorporation of the intact doubly labeled metabolites. Rather, the M + 2 peaks (via electrospray as protonated M + 1 molecular ions) were more intense than the M + 3 peaks (protonated M + 2 molecular ions), thus confirming that the double label had not been incorporated.

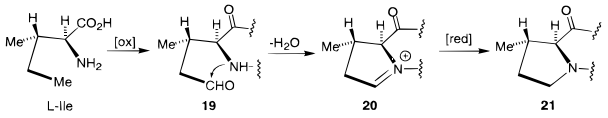

Figure 2.

Oxidative cyclization of the nitrogen atom onto the C-5-methyl group of isoleucine appears to be a unique biosynthetic transformation.[6,7] A reasonable pathway, depicted in Figure 2, would involve four-electron oxidation of the distal side chain methyl group to aldehyde **19** followed by cyclization and loss of water to produce iminium **20**; subsequent reduction (or in the case of VM55597, oxidation) of **20** furnishes the β-methylproline derivative **21**.

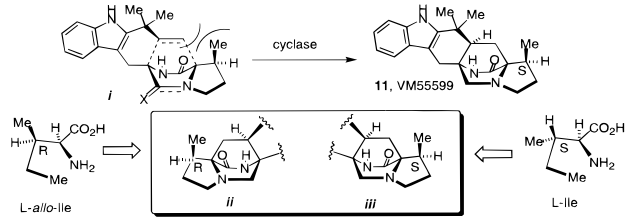

Figure 3.

Another interesting implication of the [1-^{13}C]-L-isoleucine incorporation into paraherquamide A involves the stereochemistry of the related metabolite VM55599 (**11**) isolated from the paraherquamide-producing mold *Penicillium* sp. IMI332995. Since paraherquamide A and VM55599 both possess the bicyclo[2.2.2] monoketopiperazine ring system, it seems plausible that these substances arise via a related or common [4 + 2] cycloaddition.[3] The relative stereochemistry of VM55599 was assigned[1e] by extensive ^{1}H NMR nOe studies, and, using the assumption that the absolute stereochemistry at C-20 is *S* (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 A (**1**), E (**5**), F (**8**), G (**9**), VM55596 (**6**), VM55597 (**7**), and VM55595 (**10**). On the bais of the findings reported here, 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. If L-isoleucine is also the precursor to the β-methylproline ring of VM55599, it must follow that the bicyclo[2.2.2] ring system of this compound must be *enantiomorphic* to that of the paraherquamides (see ii and iii, Figure 3). 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] ring system absolute stereochemistry as paraherquamide A (compare **1** and ii). Finally, since the methyl group of the β-methylproline ring is *syn* to the isoprene unit comprising the bicyclo[2.2.2] ring system, this implies that the cyclization to form this system occurs from the more hindered face of the azadiene system (see i, Figure 3). The stereochemical paradox posed by VM55599 raises numerous interesting questions concerning the biogenesis of these substances: is VM55599 a biosynthetic precursor to the paraherquamide family members **6**, **7**, and **9** or is VM55599 a minor shunt metabolite with the opposite absolute stereochemistry of the bicyclo[2.2.2] ring system? The mechanism of formation of the bicyclo[2.2.2] ring system in both series continues to pose[3] an interesting stereochemical and enzymological phenomenon.

Efforts are underway to resolve the stereochemical puzzle posed by VM55599 and to fully establish the sequence of events that result in modeling of the substituted proline derivatives[8] that comprise this alkaloid family.

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Supporting Information Available: Experimental procedures and spectral data for all new compounds employed in this study including methods of isotopic incorporation (8 pages). See any current masthead page for ordering and Internet access instructions.

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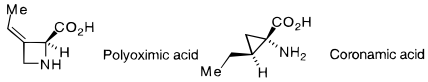

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