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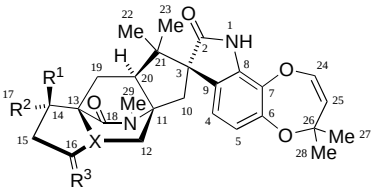

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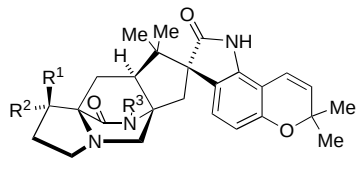

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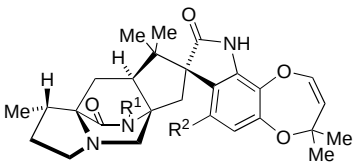

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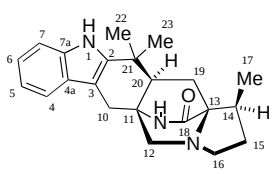


13, VM55599

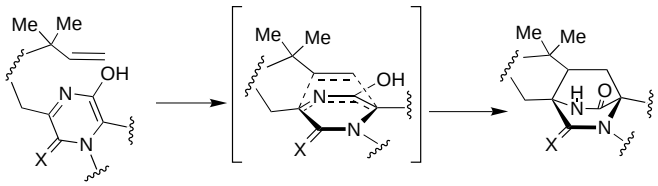
Figure 1. **1**, paraherquamide A: $R^1 = OH$, $R^2 = Me$, $R^3 = H_2$, $X = N$; **2**, paraherquamide B: $R^1 = H$, $R^2 = H$, $R^3 = H_2$, $X = N$; **3**, paraherquamide C: $R^1 = R^2 = CH_2$, $R^3 = H_2$, $X = N$; **4**, paraherquamide D: $R^1 = O$, $R^2 = CH_2$, $R^3 = H_2$, $X = N$; **5**, VM55596: $R^1 = OH$, $R^2 = Me$, $R^3 = H_2$, $X = N^+\text{-}O^-$; **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: $R^1 = H$, $R^2 = Me$, $R^3 = H$.

Studies on the Biosynthesis of Paraherquamide: Synthesis and Incorporation of a Hexacyclic Indole Derivative as an Advanced Metabolite**

Emily M. Stocking, Juan F. Sanz-Cervera, and Robert M. Williams*

The paraherquamides (Figure 1),[1] along with the brevianamides,[2] marcfortines,[3] and sclerotamides[4] are indolic fungal metabolites that share the common structural feature of an unusual bicyclo[2.2.2]diazaoctane core. It has been postulated that the bicyclo[2.2.2]diazaoctane ring system arises through an intramolecular hetero Diels–Alder cycloaddition of the isoprene moiety across the α-carbons of the amino acid subunits, as shown in Scheme 1.[5]

In 1993, Everett and co-workers isolated a very minor metabolite that also possesses the bicyclo[2.2.2]diazaoctane core, VM55599 (**13**, Figure 1), from *Penicillium* sp. (IMI 332995) which produces paraherquamide A.[6] Based on



Scheme 1. Proposed formation of the bicyclo[2.2.2]diazaoctane ring system in the paraherquamides.

the structural similarities of these co-metabolites, Everett et al. speculated that VM55599 might be a biosynthetic precursor to paraherquamide A.[6] The relative stereochemistry of VM55599 as shown in Figure 1 was assigned by ^{1}H NMR spectroscopy with nuclear Ovenhauser enhancements but the absolute configuration of this substance remains unknown. The stereochemistry of the methyl group in the β-methylproline ring was assigned as being *syn* to the bridging isoprene moiety. In all other known members of the paraherquamide family, the methyl group in the β-methylproline ring is disposed *anti* to the bridging isoprene moiety. If VM55599 was indeed a precursor to paraherquamide A, then oxidation of the β-methylproline ring would have to occur with inversion of stereochemistry at the C-14 center that bears the methyl group.

Previous studies from this laboratory on the biosynthesis of paraherquamide A demonstrated that L-isoleucine is the precursor to the β-methyl-β-hydroxy proline ring of paraherquamide A.[7] The relative disposition of the methyl group in the prolyl ring is retained in the biosynthetic conversion of L-isoleucine into paraherquamide A and, thus, the hydroxylation at C-14 occurs with net retention. These findings bring into question the potential intermediacy of VM55599 in the biosynthesis of the paraherquamides. Furthermore, if L-isoleucine is also the precursor to VM55599, then the absolute stereochemistry of this metabolite must be that depicted in

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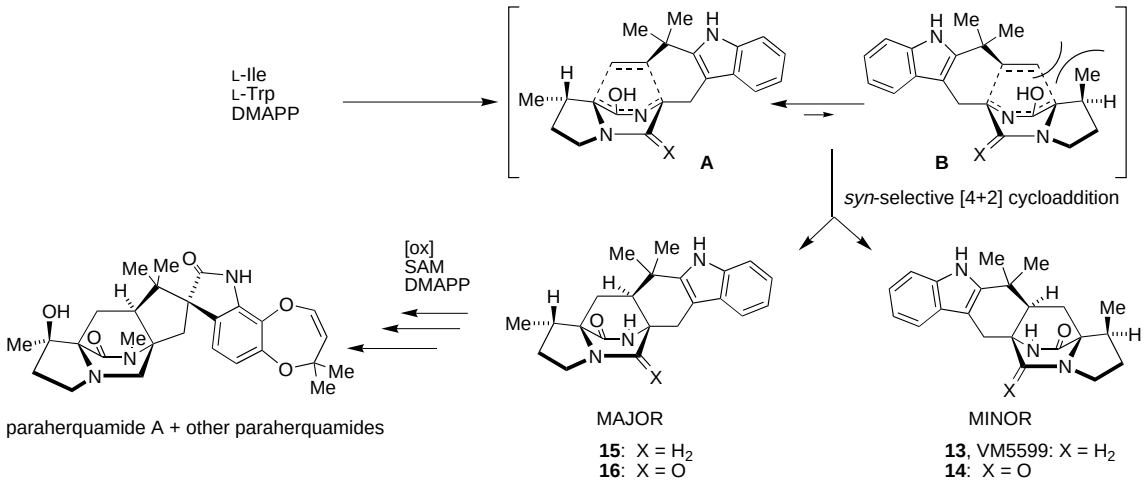


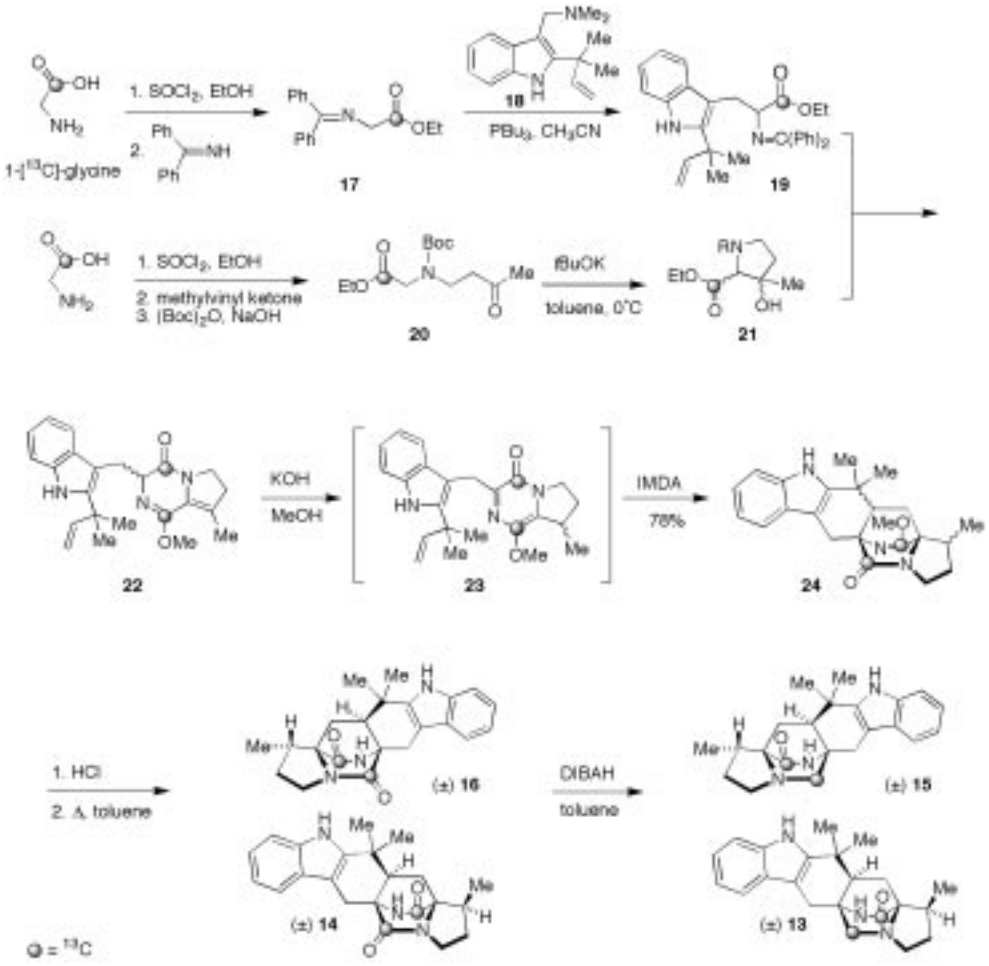
Scheme 2. Proposed unified biogenesis of VM55599 and the paraherquamides. DMAPP = dimethylallylpyrophosphate, SAM = *S*-(5′-adenosyl)-L-methionine chloride.

Figure 1 wherein the absolute configuration of the bicyclo[2.2.2]diazaoctane portion of this molecule is *enantiomorphic* to that of paraherquamide A.[8]

A unified biogenesis of VM55599 and the paraherquamides has been previously suggested as shown in Scheme 2.[9] Every metabolite would result from the intramolecular [4+2] cycloaddition of a common azadiene through two of the four possible diastereomeric transition structures. If cycloaddition occurs with the methyl group of the β-methylproline ring *anti* to the isoprene unit (as in **A**, Scheme 2), then the intermediate **15** would be formed and would lead to all of the paraherquamides containing a β-methylproline moiety. This is presumed to be the major pathway. A minor shunt pathway would involve cycloaddition from the more hindered face of the azadiene system (see **B**, Scheme 2) with the methyl group of the β-methylproline ring *syn* to the isoprene unit; VM55599 would thus result. To test this hypothesis, we have synthesized racemic doubly ^{13}C-labeled putative cycloadducts (**13**–**16**) and have examined these substances as potential pathway metabolites in *Penicillium fellutanum*.

The proposed ^{13}C-labeled compounds were synthesized according to our previously described synthesis of racemic VM55599 (Scheme 3).[9c, 10] The advantage of this strategy is that all four candidate precursors are accessible from a single synthesis. In addition, both of the ^{13}C labels in the product are derived from relatively inexpensive ^{13}C-glycine.

The racemic cycloadducts (**13**–**16**) possessed the unfortunate property of being insoluble in water so rendering the planned feeding experiments challenging. To circumvent this problem, the detergent TWEEN 80 was added and was found



Scheme 3. Synthesis of the ^{13}C-labeled compounds **13**–**16**. Boc = *tert*-butoxycarbonyl, IMDA = *N*-(carboxymethyl)glycine, DIBAH = diisobutylaluminum hydride.

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to increase the miscibility of these substances in the culture broth without inhibiting production of paraherquamide A. Feeding experiments were performed on *P. fellutanum* (ATCC 20841) with all four potential precursors, followed by isolation and purification of paraherquamide A. Within the limits of detection by ^{13}C NMR spectroscopy and mass spectrometry no incorporation was observed for VM55599 ((±)-**13**) or its oxidized counterpart (±)-**14**. In addition, no incorporation was observed for the diketopiperazine (±)-**16**. However, for the C-14 epimer of VM55599 ((±)-**15**), significant incorporation was observed by ^{13}C NMR spectroscopy at C-12 and C-18 of paraherquamide A. From analysis of the electrospray mass spectrum, incorporation was determined to be 0.72% for the intact doubly labeled material.[11] ^{13}C-Monolabeled paraherquamide A, from catabolism of (±)-**15**, was not detected in the mass spectrum. The implications of these observations are considerable.

Since the diketopiperazine (±)-**16** was not incorporated, this raises interesting questions concerning the timing of the reduction of the prolyl-derived carbonyl group. The incorporation of compound (±)-**15** in significant isotopic yield, indicates that the formation of the bicyclo[2.2.2]diazaoctane occurs at the stage with the nonoxidized tryptophyl moiety (that is, the indolyl group). This mandates that oxidations of the indole ring to form both the catechol-derived dioxepin and spirooxindole occur *after* the formation of this intermediate. It thus follows that the dioxepin-derived isoprenylation and the *S*-adenosylmethionine-mediated *N*-methylation reactions occur late in the pathway. These results also cast considerable doubt on the intermediacy of VM55599[6] and its oxidized precursor **14** in the paraherquamide biosynthesis and provide additional circumstantial evidence that VM55599 is a minor shunt metabolite. Finally, the present work documents the intermediacy of an advanced metabolite **15**, which contains the core structural elements of the paraherquamide framework, prior to a series of oxygenation reactions. Efforts to elucidate the exact sequence of biosynthetic reactions immediately preceding and following the formation of **15** are currently under way in these laboratories.

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[8] Elucidation of the absolute stereochemistry of VM55599 is in progress. The absolute stereostructures for compounds **13**–**16** (which are racemic) are depicted with the absolute configurations presumed to be those of the natural metabolites.

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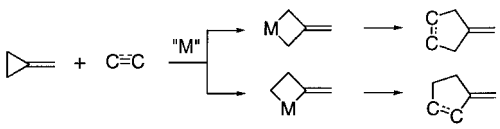
Novel [3+2] Cycloaddition of Alkylidenecyclopropanes with Aldehydes Catalyzed by Palladium

Itaru Nakamura, Byoung Ho Oh, Shinichi Saito, and Yoshinori Yamamoto*

A metal-catalyzed cycloaddition between methylenecyclopropane and a carbon–carbon multiple bond can proceed through two different reaction pathways to give regioisomeric [3+2] carbocycles (Scheme 1).[1–3] The research groups of



Scheme 1. Metal-catalyzed cycloaddition of methylenecyclopropane and a carbon–carbon multiple bond.

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