Studies on the Biosynthesis of Paraherquamide: Concerning the Mechanism of the Oxidative Cyclization of L-Isoleucine to β-Methylproline

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Paraherquamide A (**1**) is one member of a group of heptacyclic fungal metabolites (**1**−**13**, Figure 1) with potent anthelminthic activity isolated from various *Penicillium* sp.[1] Among its unusual structural features, paraherquamide A contains a β-methyl-β-hydroxy proline moiety. A previous report from this laboratory on the biosynthesis of paraherquamide A demonstrated that the prolyl ring is formed via a heretofore-unknown oxidative cyclization of the terminal methyl group of L-isoleucine onto the α-amino group.[2,3]

There are several possibilities of how the oxidative cyclization of L-isoleucine to β-methylproline can occur; two reasonable prospects are shown in Scheme 1. One pathway involves 4-electron oxidation of the distal side-chain methyl group to aldehyde **15** followed by cyclization and loss of water to produce the imine **16**; subsequent reduction of **16** (or in the case of VM55597, oxidation) furnishes the β-methylproline ring, **17**. Another reasonable pathway is oxidation of the terminal methyl group to an alcohol (**18**, R = H) followed, for example, by phosphorylation and nucleophilic displacement of the phosphate group. One other possibility is chlorination of the distal side chain methyl group to **19** followed by nucleophilic displacement to give **20**. Precedent for the latter pathway was reported by Arigoni and Looser, where chlorinated leucine moieties in the natural product victorin C were observed.[4] To help distinguish between $2e^-$ (via **18** or **19**) and $4e^-$ (via **15**) oxidation mechanisms in these putative pathways, L-[5-^{13}C,5-^{2}H$_3$]isoleucine was synthesized and fed to cultures of *P. fellutanum* (ATCC: 20841). Depending on the net oxidation state change, the labeling pattern shown in **17** or **20** should be observed for the $4e^-$ and $2e^-$ pathways in the isolated paraherquamide A, respectively.

L-[5-^{13}C,5-^{2}H$_3$]Isoleucine was synthesized by using the procedure developed by Oppolzer and co-workers for the synthesis of unlabeled L-isoleucine (Scheme 2).[5,6] However, since 2-[^{13}C^{2}H$_3$]-ethylmagnesiumbromide is not commercially available, it was synthesized from ^{13}C^{2}H$_3$-iodomethane.[7] Reaction of thioanisole

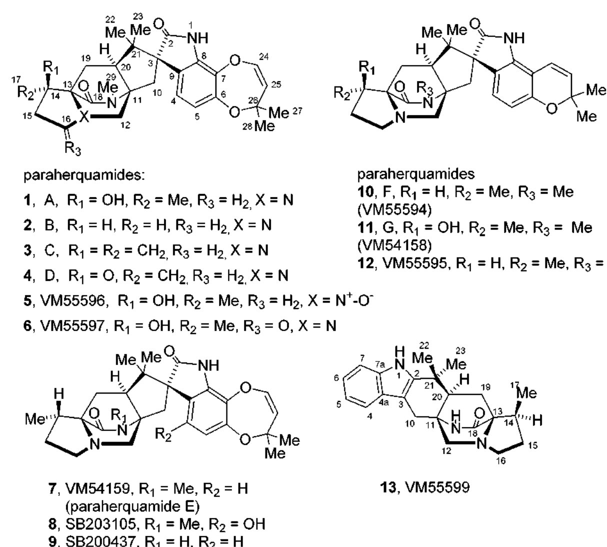
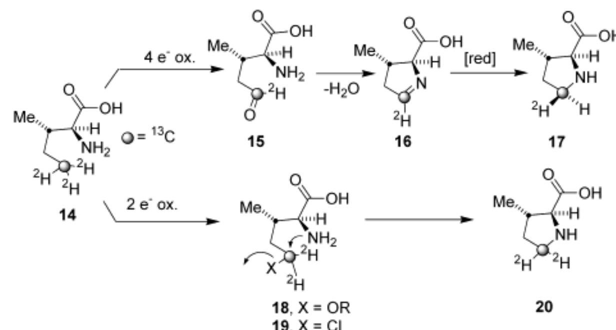
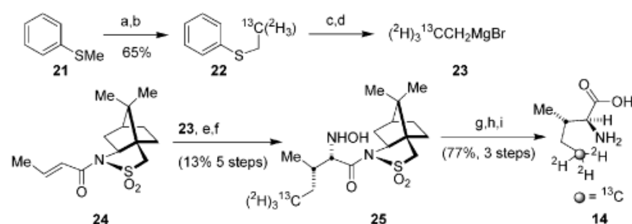


Figure 1.

Scheme 1. Possible Pathways for the Oxidative Cyclization of L-Isoleucine to β-Methylproline



Scheme 2. Synthesis of l-[5-^{13}C,5-^{2}H$_3$]Isoleucine[a]



[a] Conditions: (a) *n*-BuLi, (b) ^{13}C(^{2}H)$_3$I, (c) benzyl bromide, 150 °C, (d) Mg^0, (e) 1-chloro-1-nitrosocyclohexane, (f) 1 N HCl, (g) Zn^0, 1 N HCl/AcOH, (h) LiOH, (i) DOWEX ion exchange.

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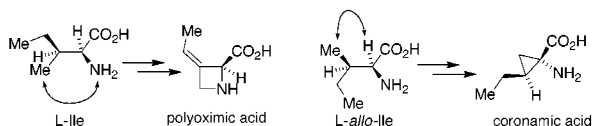
(1) (a) Yamazaki, M.; Okuyama, E.; Kobayashi. M.; Inoue, H. *Tetrahedron Lett.* **1981**, *22*, 135−136. (b) Ondeyka, J. G.; Goegelman, R. T.; Schaeffer, J. M.; Kelemen, L.; Zitano, L. *J. Antibiot.* **1990**, *43*, 1375−1379. (c) Liesch, J. M.; Wichmann, C. F. *J. Antibiot.* **1990**, *43*, 1380−1386. (d) Blanchflower, S. E.; Banks, R. M.; Everett, J. R.; Manger, B. R.; Reading, C. *J. Antibiot.* **1991**, *44*, 492−497. (e) Blanchflower, S. E.; Banks, R. M.; Everett, J. R.; Reading, C. *J. Antibiot.* **1993**, *46*, 1355−1363.

(2) Stocking, E. M.; Sanz-Cervera, J. F.; Williams, R. M.; Unkefer, C. J. *J. Am. Chem. Soc.* **1996**, *118*, 7008−7009.

(3) There are other known oxidative cyclizations of L-isoleucine. The β-methyl group of L-isoleucine has been postulated to undergo oxidative cyclization onto the amino terminus to form the heterocycle, polyoximic acid. (Hanessian, S.; Fu, J.-M.; Tu, Y.; Isono, K. *Tetrahedron Lett.* **1993**, *43*, 4153−4156.) In L-*allo*-isoleucine, the β-methyl group is know to undergo oxidative cyclization onto the α-carbon to form coronamic acid. (Pany, R. J.; Mhaskar, S. V.; Lin, M. T.; Walker, A. E.; Mafoti, R. *Can. J. Chem.* **1994**, *72*, 86−99.)



(4) (a) Looser, M. Ph.D. Thesis, ETH, 1989 (D. Arigoni). (b) An alternative pathway for β-methylproline biosynthesis has been discovered in the biosynthesis of the antibiotic bottromycin in *Streptomyces bottropenis* involving an *S*-adenosylmethionine-based β-methylation of proline, see: Kellenberger, J. L. Ph.D. Thesis, ETH, 1997 (D. Arigoni).

(5) (a) Oppolzer, W.; Tamura, O. *Tetrahedron Lett.* **1990**, *31*, 991−994. (b) Oppolzer, W.; Tamura, O.; Deerberg, J. *Helv. Chim. Acta* **1992**, *75*, 1965−1973.

(6) Yields shown reflect nonoptimized reaction conditions.

(7) The synthesis of this substance will be reported elsewhere, but is also commercially available.

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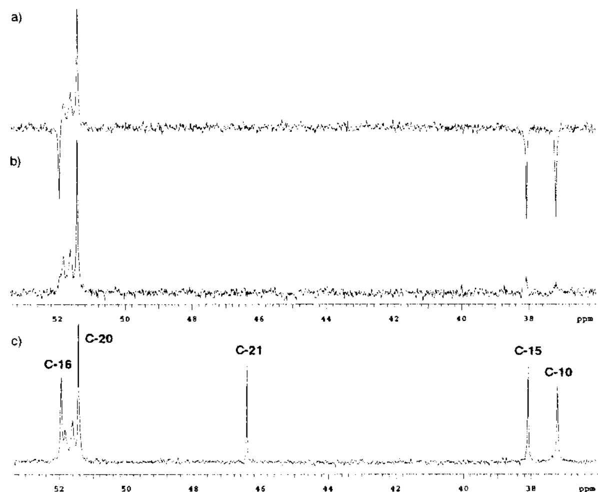

Figure 2. (a) DEPT 135 experiment with CH up and CH_2 down, (b) DEPT 90 experiment with only CH's shown, and (c) partial ^{13}C spectrum (100 MHz) of **1** from the feeding experiment with 5-[$^{13}C^2H_3$]-L-ile.

(**21**) with *n*-BuLi followed by the addition of [$^{13}C^2H_3$]-iodomethane provided $^{13}C^2H_3$-ethylphenylsulfide (**22**). The 2-[$^{13}C^2H_3$]-ethylbromide, distilled from the reaction of **22** with benzylbromide at 150 °C, was added dropwise as an ethereal solution to activated Mg^0 to form the Grignard reagent, **23**. Successive treatment of the *N*-crotonoylborane-10,2-sultam (**24**) with 2-[$^{13}C^2H_3$]-ethylmagnesiumbromide followed by 1-chloro-1-nitrosocyclohexane and 1 N aqueous HCl at −78 °C provided the 1,4-addition/electrophilic amination product (**25**). *N,O*-Hydrogenolysis of the hydroxylamine, **25**, with Zn^0 powder in 1 N HCl/AcOH, followed by saponification of the sultam with LiOH in THF/H_2O and DOWEX ion exchange, provided L-[5-^{13}C,5-$^2H_3$]isoleucine (**14**).

A feeding experiment in *Penicillium fellutanum* with L-[5-^{13}C, 5-$^2H_3$]isoleucine (**14**), followed by isolation and purification of the paraherquamide A produced, revealed 0.32% incorporation of the labeled amino acid.[8] Close inspection of the ^{13}C-spectrum (Figure 2c) revealed a triplet at 51.6 ppm, which would indicate that, in the labeled compound, C-16 is coupled to a single deuterium atom. However, as seen in Figure 2c, the triplet was partially obscured by neighboring ^{13}C-signals, which complicated interpretation of the spectrum. To resolve this problem, DEPT experiments were performed.

As seen in Figure 2, the DEPT spectra unambiguously assigns a $^{13}C^2H^1H$ pattern to C-16 in the labeled paraherquamide A. In both the DEPT 135 and DEPT 90 spectra, the triplet is seen. In the event of a $^{13}C^2H_2$ pattern, the triplet would not appear in either DEPT spectrum. *From these experiments, it was determined that cyclization of L-isoleucine occurs though a 4e$^-$ oxidation of the terminal methyl group such as via the putative intermediate **15** followed by cyclization and diastereoselective 2e$^-$ reduction to give **17**.*

To determine if the *pro-R* or *pro-S* hydrogen was retained in the oxidative cyclization, CW-selective proton decoupling experiments were performed as shown in Figure 3. The ^{13}C signals were decoupled from protons H-16a (*pro-S*) at 3.21 ppm (Figure 3c) and H-16b (*pro-R*) at 2.22 ppm (Figure 3b), respectively.[9] When H-16a is decoupled (Figure 3c), the expected triplet splitting

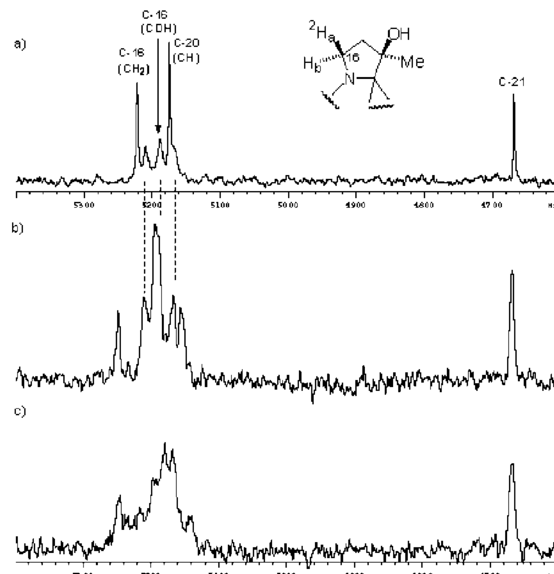

Figure 3. (a) Partial ^{13}C spectrum (100 MHz) of paraherquamide A (**1**) from the feeding experiment with 5-[$^{13}C^2H_3$]-L-ile, (b) partial ^{13}C spectrum (100 MHz) of **1** from the experiment with H-16b at 2.22 ppm decoupled, and (c) partial ^{13}C spectrum of **1** (100 MHz) from the experiment with H-16a at 3.21 ppm decoupled.

pattern (seen in Figure 3a) for the deuterium coupled ^{13}C-labeled C-16 becomes complex suggesting that $^{13}C^2H$ is coupled to H-16b. When H-16b is decoupled (Figure 3b), the triplet from the deuterium coupled ^{13}C signal is not affected. Therefore, it was determined that H-16a (3.21 ppm) is the deuteron, H-16b (2.22 ppm) is the proton and the *pro-S* hydrogen is retained in the oxidative cyclization.[10] *This result implies that reduction occurs on the same face of the proline ring as the methyl group, C-17.*

In summary, the results described provide the first mechanistic glimpse of the events likely to be involved in the biosynthesis of the β-methylproline moiety of the paraherquamide family of anthelmintic agents. Efforts to elucidate the exact nature of the enzymatic oxidizing and reducing species involved in the conversion of L-isoleucine to β-methylproline are under investigation in these laboratories.

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Supporting Information Available: Full experimental procedures for the synthesis of L-[5-^{13}C,5-$^2H_3$]isoleucine, method for the determination of the percentage of isotopic incorporation, and HSQC spectrum of **1** from the feeding experiment with $^{13}C^2H_3$-L-ile (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Determined from the ES mass spectrum. Specific incorporation at C-16 is 0.21% as determined by NMR. We have shown good incorporation of 1-[^{13}C]-L-Ile in the past (see ref 2), but the deuterium isotope effect is expected to have an effect on the rate of biosynthesis of β-methylproline. This can account for both the low percentage of incorporation and the relatively low yield of **1** isolated in this experiment.

(9) Assignments of H-16a and H-16b are based on the assignments of Blanchflower and co-workers (refs 1d and 1e).

(10) This result was confirmed through a HSQC experiment. The ^{13}C signal from the deuterium-coupled C-16 shows connectivity with the proton at 2.22 ppm, H-16b, but not with the proton at 3.21 ppm, H-16a.