Processing 2-Methyl-L-Tryptophan through Tandem Transamination and Selective Oxygenation Initiates Indole Ring Expansion in the Biosynthesis of Thiostrepton

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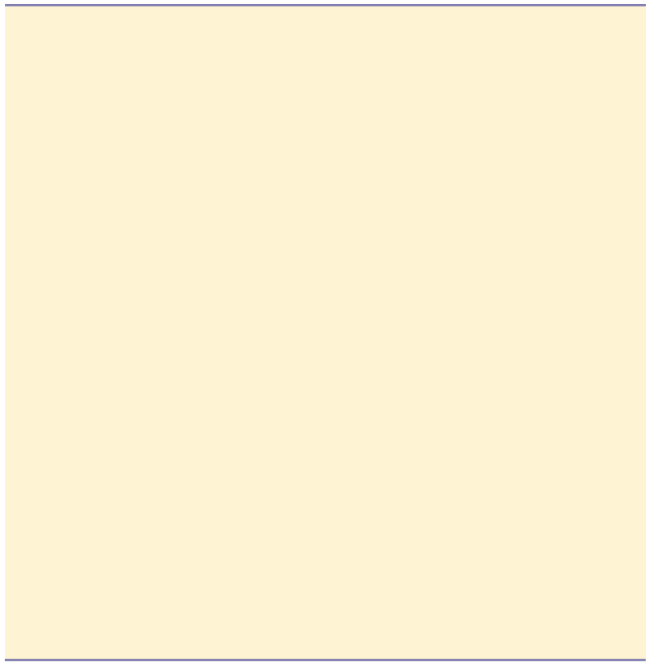

chain of L-Trp, i.e., the removal of the C2′−N unit and a shift of the carboxylate group onto the indole ring, and produces 3-methyl-2-indolic acid for further incorporation. In contrast, the process through which QA is formed remains poorly understood. This process results in more complex changes in the structure of L-Trp, including ring expansion by cleavage of the C2−N1 bond for indole ring opening and the connection of C2′ of the carbon side chain to N1 for recyclization.[7b] Focusing on this key transformation, we here dissect an unusual mechanism for indole ring expansion and demonstrate that processing the precursor 2-methyl-L-Trp through tandem transamination and selective oxygenation triggers an intramolecular rearrangement 7bFocusing

process, a shunt product was proposed to arise from the decarboxylation of an uncharacterized oxygenated intermediate during the formation of **1**. Two related 2-methyl-indole derivatives, α-hydroxyl carboxylate **4** from Route 2 and lactone **5** from Route 4 (Figure 3), were then synthesized and served as standards for structural determination (Supplementary Results). High-performance liquid chromatography with mass spectrometric detection (HPLC-MS) indicated that this product differs from **4**. Instead, it is identical to **5**, leading to a hypothesis that TsrE-catalyzed oxygenation directly targets the pyrrole part of the indole ring. Furthermore, TsrE was found to hydroxylate 2-methyl-3-propyl-indole (**6**, a synthetic mimic of **3**, $[M + H]^+$ m/z: calcd. 174.1277 for $C_{12}H_{16}N$, found 174.1278) at C3 and trigger a double bond shift within the pyrrole ring, yielding an imine product, **7** ($[M + H]^+$ m/z: calcd. 190.1226 for $C_{12}H_{16}NO$, found 190.1226) (Figure 4B). Chiral separation of **7** on HPLC and subsequent electronic circular dichroism (ECD) calculation validated the stereoselectivity of TsrE (Figures S6 and S7), which produced a (3*S*)-isomer with an enantiomeric excess (*ee*) value of up to 96%. Using $^{18}O_2$ in the TsrE-catalyzed hydroxylation of **6** led to the production of single ^{18}O-labeled **7** ($[M + H]^+$ m/z: calcd. 192.1269 for $C_{12}H_{16}N^{18}O$, found 192.1265). Consequently, these observations provide strong evidence that TsrE can catalyze the selective oxygenation of **3** and generate (*S*)-3-(3-hydroxy-2-methyl-3H-indol-3-yl)-pyruvic acid (**8**) (Figure 3), which exists in an imine form and appears to be too unstable to be detected at 30 °C.

By lowering the incubation temperature, we slowed the transformation of the precursor **2** that involves both TsrA and TsrE activities. Remarkably, the reaction proceeding at 4 °C did accumulate **8** ($[M - H]^-$ m/z: calcd. 232.0615 for $C_{12}H_{10}NO_4$, found 232.0612), which results from 3-hydroxylation (or **8′** from 2,3-epoxidation, an oxygenation alternative that cannot be completely excluded at this time because the facile tautomerization between **8** and **8′** could occur in solution), as judged by careful HPLC-high resolution (HR)-MS and MS/MS analyses (Figure S8). At this temperature, using $^{18}O_2$ in the reaction mixture produced single ^{18}O-labeled **8** (or **8′**, $[M - H]^-$ m/z: calcd. 234.0658 for $C_{12}H_{10}NO_3{}^{18}O$, found 234.0659), consistent with the notion that TsrE-mediated indole ring expansion is initiated by a cryptic selective oxygenation of **3** rather than dehydrogenation (Route 1) or halogenation (Route 3). These analyses revealed a set of related derivatives, further supporting the following conversion process toward the formation of **1** through Route 4 (Figures 3 and S8). Intermediate **8** (or **8′**), which possesses a highly reactive pyrrole imine ring, could be

readily hydrated to generate **9** ($[M - H]^-$ m/z: calcd. 250.0721 for $C_{12}H_{12}NO_5$, found 250.0721; and calcd. 252.0763 for $C_{12}H_{12}NO_4{}^{18}O$, found 252.0757), an extremely unstable 2,3-dihydroxylated pyrroline intermediate. Breaking the N1−C2 bond of **9** would result in intermediate **10** ($[M - H]^-$ m/z: calcd. 250.0721 for $C_{12}H_{12}NO_5$, found m/z 250.0720; and calcd. 252.0763 for $C_{12}H_{12}NO_4{}^{18}O$, found 252.0766). Ring expansion relies on the condensation occurring between the released amino group and the α-keto group of **10** to produce a recyclized intermediate, which would undergo dehydration/aromatization to form quinoline ketone **1**. Alternatively, intermediate **8** could undergo the oxidative decarboxylation of its carbon side chain (particularly in the presence of O_2 and $FADH_2$, which react to form the oxidizing adduct FAD-4a-OOH), and the subsequent nucleophilic attack of the newly generated carboxylate group onto C2 would furnish an indole lactone to produce shunt product **5** (Figure S9). The production of double ^{18}O-labeled **5** ($[M - H]^-$ m/z: calcd. 208.0751 for $C_{11}H_{12}NO^{18}O_2$, found 208.0749) in the presence of $^{18}O_2$ supported this conversion. The oxidative decarboxylation of the carbon side chain appears to compete with the process of indole ring expansion, and the yield of **5** evidently increased with the decrease in incubation temperature (Figure S5).

In conclusion, we demonstrate that the formation of quinoline ketone intermediate **1** in TSR biosynthesis involves the tandem activities of TsrA and TsrE for processing the precursor 2-methyl-L-Trp (**2**) through reversible transamination and selective oxygenation. TsrA, a flexible PLP-dependent aminotransferase, activates C2′ of the carbon side chain through transamination to generate α-keto acid **3**. TsrE, a flavin-dependent protein, selectively oxygenates **3** to produce a highly reactive indole imine, which then undergoes a rearrangement process through intermediates **9** and **10** for ring expansion. More likely, this unstable intermediate is C3(*S*)-hydroxylated **8** according to data presented here and a similar intermediate resulting from unusual FAD-dependent protein activity, which initiates indoloterpenoid cyclization through a selective C3-

23(gener8ted)-422.6(3-6vatesa)0(n)-5F10-362121Tf16.809301.3848c(2)Tj/F71Tf.46490TD78