Biosynthetic Mechanism

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Mimicking Dimethylallyltryptophan Synthase: Experimental Evidence for a Biosynthetic Cope Rearrangement Process**

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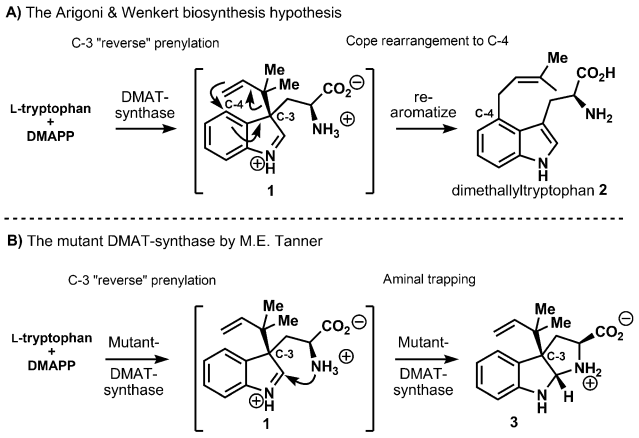
Dedicated to Professor Johann Mulzer on the occasion of his retirement

Prenylation of the indole nucleus is a fundamental process in the biosynthesis of alkaloids.[1] This step is catalyzed by various prenyltransferases, which direct the reaction to different sites of the indole core. Thereby a dimethylallyl carbocation reacts in an electrophilic aromatic substitution reaction at the indole nucleus,[2] leading either to a normal prenylation product (attack at the primary position of the dimethylallyl cation) or a reverse prenylation product (attack at the tertiary position).[3] The normal prenylation at C-4 of tryptophan is catalyzed by dimethylallyltryptophan (DMAT) synthase, and comprises the first step in the biosynthesis of ergot alkaloids. This process is especially noteworthy as the enzyme forms a C–C bond at the least nucleophilic position (C-4) of the indole core—instead of the highly nucleophilic positions C-2 and C-3.[4] One possible explanation for this observation was given by Arigoni and Wenkert, and is displayed in Scheme 1 A.[5]

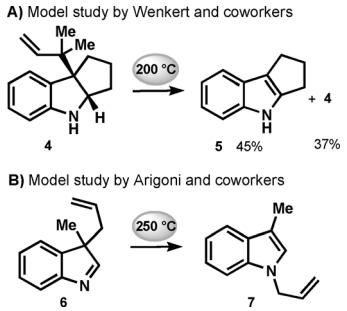
In their hypothesis, an initial reverse prenylation of tryptophan at C-3 with dimethylallyl pyrophosphate (DMAPP) was postulated to give compound **1**.[6] Subsequent Cope rearrangement shifts the prenyl moiety to the C-4 position and re-aromatization gives dimethylallyltryptophan **2**. Evidence favoring this hypothesis was recently provided by Tanner et al., with a catalytically active mutant DMAT-synthase (FgaPT2), producing tricyclic compound **3** instead (Scheme 1 B).[7c] Compound **3** is clearly obtained by way of reverse prenylation, and consecutive aminal formation.

As examples of enzymes catalyzing pericyclic reactions are rare,[1c,5a,b,6a,8,9] the Arigoni and Wenkert biosynthetic hypothesis was dismissed because the chemical feasibility of a sigmatropic prenyl-shift could not be shown in vitro. Model compounds **4** and **6** (Scheme 2 A,B) failed to give C-4 rearrangement products, but provided products **5** and **7** instead.[5b,c]

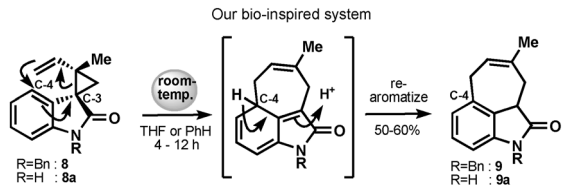
Herein, we report the first experimental evidence to support the Cope rearrangement hypothesis as a possible biosynthetic pathway, and show the synthetic feasibility of this chemical transformation. The reaction proceeds at room temperature and is not dependent on the solvent (Scheme 3). This led us to conclude that the enzyme might catalyze the reaction by forcing the substrate into the reactive conformation.



Scheme 1. The Arigoni and Wenkert biosynthetic hypothesis.



Scheme 2. Model systems **4** and **6** that failed to undergo the Cope rearrangement to the C-4 position.



Scheme 3. The bio-inspired [3,3]-sigmatropic rearrangment of **8** and **8a**. THF = tetrahydrofuran; PhH = benzene; Bn = benzyl.

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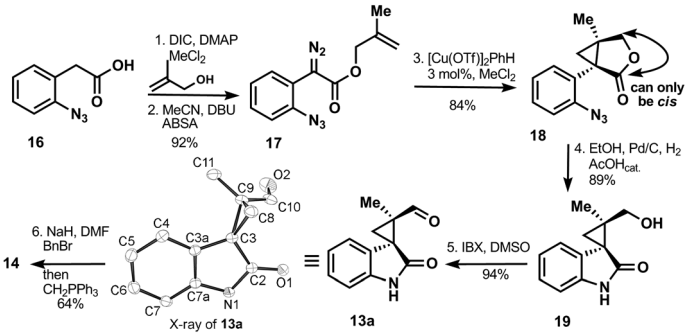
Our bio-inspired system **8** mimics the conformational restrictions the enzyme likely imposes on the substrate when pre-orienting it in the active site. It contains a spiro-fused vinyl-cyclopropane ring, responsible for the rigidity of the system (Scheme 3). Therefore, the stereochemistry of the vinyl group pointing towards the benzene ring is crucial for orbital overlap. The methyl group of **8** is resembles that of **1** and facilitates the Cope rearrangement (divinylcyclopropane rearrangement in this case) through a *gem*-dimethyl effect.[10] We think these architectural features account for the successful rearrangement of **8** even at room temperature. Compounds **4** and **6**, which failed to undergo rearrangement, are very flexible, and **6** lacks a *gem*-dimethyl group.[5b,c] Our system **8** deviates from bio-system **1** with respect to the oxidation state of the indole nucleus, but at the same time displays identical geometrical properties namely sp^2 hybridization at respective carbon atoms. To show that the deviations in electronic nature do not hamper a [3,3]-sigmatropic rearrangement, **8a**, without a protecting group, was rearranged to give **9a** in good yields (Scheme 4A). Additionally, **8b**[11] was reduced with sodium borohydride in methanol to give rearranged indol **15** in one pot (Scheme 4B). Indole **15** exhibits the same oxidation state as bio-system **2**.

Synthesis of **8** started with a cyclopropanation of **11** with diazo-isatin **10**.[12,13] Thereby, two separable diastereomers were formed in a 1:1 ratio in 60% combined yield, following deprotection of the TBS-group and subsequent oxidation of aldehydes **12** and **13**. The Wittig reaction afforded diastereomeric olefins **8** and **14**, of which **8** was ideally suited for the rearrangement, whereas diastereomer **14** cannot undergo the sigmatropic rearrangement (no orbital overlap of the vinyl group with the aromatic ring). Indeed only one diastereomer afforded tricycle **9**. Therefore we developed a stereoselective route to **14** to unambiguously assign the structures of **8** and **14**.

For this purpose, azido-acid **16** was converted to **18** in three steps.[14,15] This sequence secured the *cis*-annelation at the two vicinal quaternary carbon centers (curved arrows in Scheme 5). The relative configuration corresponds to diastereomer **14** and was confirmed by single-crystal X-ray analysis of **13a**.[16] Reduction of the azide and cyclization delivered oxindole **19** in 89% yield. Oxidation of **19** followed by protection of the lactam with benzyl bromide and consecutive Wittig reaction afforded diastereomer **14**.



Scheme 5. Synthetic route to diastereomer **14**. DIC = *N*,*N*-diisopropylcarbodiimide; DMAP = 4-*N*,*N*-dimethylaminopyridine; DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; ABSA = *p*-acetamidobenzenesulfonyl azide; DMSO = dimethylsulfoxide; DMF = *N*,*N*-dimethylformamide; OTf = triflate.

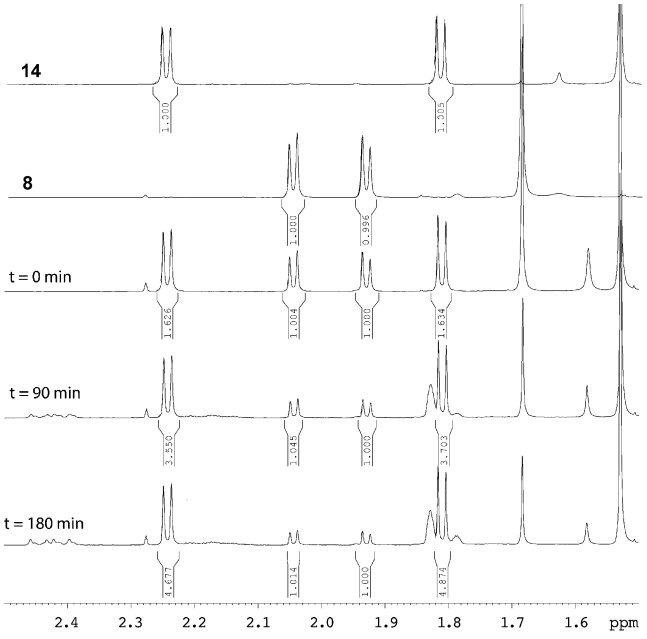
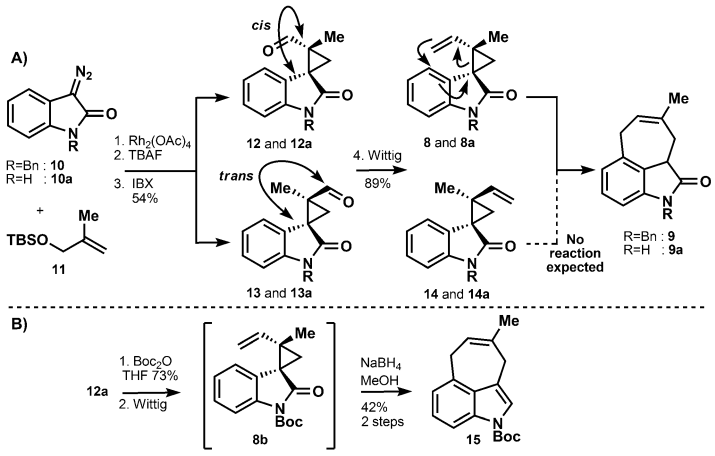


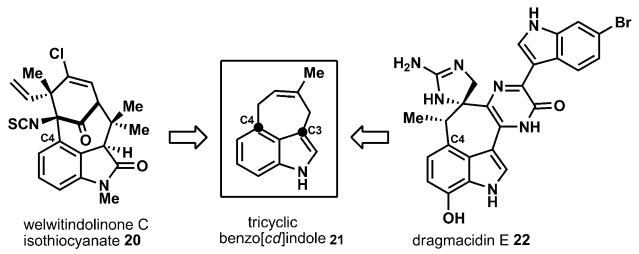
Figure 1. Reactivity of **8** in contrast to **14** at 60 °C as monitored by NMR spectroscopy.



Scheme 4. Synthesis of the bio-inspired system **8** (A) and **15** (B). TBAF = tetrabutylammonium fluoride; IBX = 2-iodoxybenzoic acid; TBS = *tert*-butyldimetylsilyl; Boc = *tert*-butyloxycarbonyl.

Spectral correlation revealed, that exclusively diastereomer **8** had rearranged to tricycle **9** at room temperature, whereas **14** did not undergo rearrangement. To visualize the reactivity of **8** a mixture of both diastereomers was subjected to NMR spectroscopy at 60 °C. The NMR signals of the CH_2-group on the cyclopropane ring were chosen as indicators of the rearrangement. As seen in Figure 1, **8** reacts whereas **14** remains essentially unchanged.

Apart from the implications of our results on the mechanism of the enzyme, our reaction provides rapid



Scheme 6. Natural products exhibiting a tricyclic benzo[*cd*]indole core.

access to indole alkaloids with a tricyclic benzo[*cd*]indole core **21** (Scheme 6), such as welwitindolinones **20** and dragmacidin E **22**.[17,18] Their syntheses are currently being pursued by our research group.

In conclusion, we provided the first experimental evidence for a possible enzyme-catalyzed sigmatropic process in the C-4 prenylation of indole alkaloids. This synthetic method allows for direct C–C bond formation with facile synthetic access to welwitindolinones **20** and dragmacidin E **22**.

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