

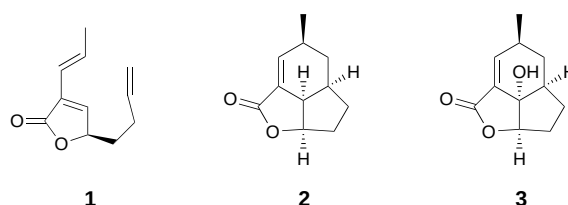
Cyclization of (–)-Pregaliellalactone in the Fungus *Galiella rufa*

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The Diels–Alder pericyclic [4+2] cycloaddition between a diene and a dienophile is one of the most powerful carbon–carbon bond forming reactions in organic chemistry,^[1] and it has been utilized in the total syntheses of many natural products.^[2] However, Nature does not appear to have appreciated the reaction to the same extent as synthetic chemists: only few examples of enzyme-catalyzed Diels–Alder reactions in biosynthetic pathways have been reported.^[3] Some secondary metabolites may be Diels–Alder adducts although it has been difficult to determine whether they were formed through an actual [4+2] cycloaddition or through an ionic or stepwise process.^[4] In reaction pathways which comprise a Diels–Alder reaction the question whether the reaction is spontaneous or catalyzed, for example by a Diels–Alderase, still remains. The problems to demonstrate the action of a Diels–Alderase in a biosynthetic pathway are associated with the difficulties to establish such a pathway unambiguously and with the low availability of biosynthetic precursors for testing.

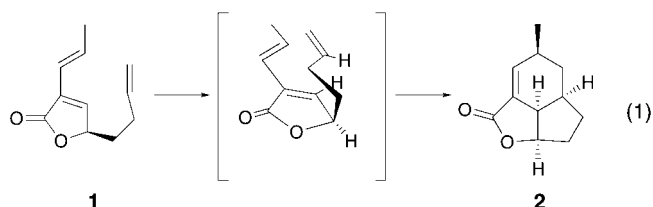
So far, only two enzyme-catalyzed biosynthetic Diels–Alder reactions have been reported: Oikawa et al. showed in several experiments that cell-free extracts from the fungus *Alternaria solani* catalyze the conversion of the linear triene precursor prosolanapyrone III to the bicyclic product solanapyrone A,^[5] but they did not characterize the enzyme. Recently, the group of Vederas showed that isolated lovastatin nonaketide synthase, LNKS, involved in the biosynthesis of the fungal metabolite lovastatin catalyzes the *exo*-selective cyclization of a hexaketide triene.^[6] The remarkable effect of the enzyme was not that it increased the reaction rate, but that it stabilized the *exo* transition state giving the opposite diastereomer compared to the spontaneous cyclization. The *exo* isomer could not be formed by chemical methods.

(–)-Galiellalactone (**3**) is a fungal metabolite isolated from the ascomycetes *Galiella rufa* (strains A75-86 and A111-95) as a highly selective and potent inhibitor of interleukin-6 (IL-6) signalling in HepG2 cells.^{[7], [8]} Biosynthetic studies and the structure of the co-metabolites (–)-**1** and (+)-**2**^[9] suggested that **3** is formed in vivo through an intramolecular Diels–Alder reaction.^{[10], [11]} When (–)-pregaliellalactone (**1**) or (+)-desoxygaliellalactone (**2**) are fed to the mycelium of the fungus *Galiella rufa* they are converted to (–)-galiellalactone (**3**),^[12] showing that, in vivo, the conversion of **1** to **3** takes



place in two discrete steps. Here we wish to discuss the first step, the cyclization, which, to our knowledge, is the first example of an intramolecular Diels–Alder reaction with inverse electron demand in a polyketide biosynthetic pathway that shows a biological rate enhancement.

Initial experiments showed that the cyclization of (–)-**1** to (+)-**2** can proceed spontaneously. The reaction was found to be very sluggish in organic solvents at room temperature. When (–)-**1** was left in a chloroform solution, the cyclized product (+)-**2** could only be detected after weeks. When (–)-**1** in toluene was heated in a sealed tube at 140 °C the conversion to (+)-**2** was complete after 5 h [Eq. (1)]. As it is known that



the rate of Diels–Alder reactions can increase in aqueous media due to the hydrophobic effect,^[13] reaction (1) was also performed in water: compound **1** cyclized at room temperature cleanly to **2**, with a half-life of **1** of 65 h. When the cyclization was conducted in a 4.86 M LiCl solution the rate was further increased (approximately twice) due to the “salting-out” effect.^[13] Spontaneous cyclization yielded only one isomer—natural (+)-desoxygaliellalactone (**2**) with all bridgehead hydrogen atoms on the same side of the ring system. No product from an *exo* transition state was ever observed, and the reaction is completely diastereoselective. Taken together, the absence of by-products and the rate enhancement in water as well as the effect of high concentrations of salt show that the cyclization of (–)-**1** to (+)-**2** is in fact a Diels–Alder reaction.

A series of experiments in which natural (–)-**1** was fed to the mycelium of *Galiella rufa* were conducted. The fungus was grown in a normal growth medium for 10–14 days, and when the secondary metabolism started, the mycelium was filtered off and washed thoroughly with saline to remove the growth medium. The washed mycelium was re-dispersed in saline and (–)-**1** dissolved in MeOH (0.1 %) was added, whereafter the mycelium was incubated at 24 °C. Compared with an abiotic control experiment (only **1** in saline), a rate enhancement in the production of the Diels–Alder adduct was observed in the presence of living mycelia ($k = 0.0015 \text{ min}^{-1}$), and the concentration of **1** was halved in only 9 h with the conditions used in this investigation (both experiments are summarized in Figure 1). The rate of the spontaneous abiotic reaction in

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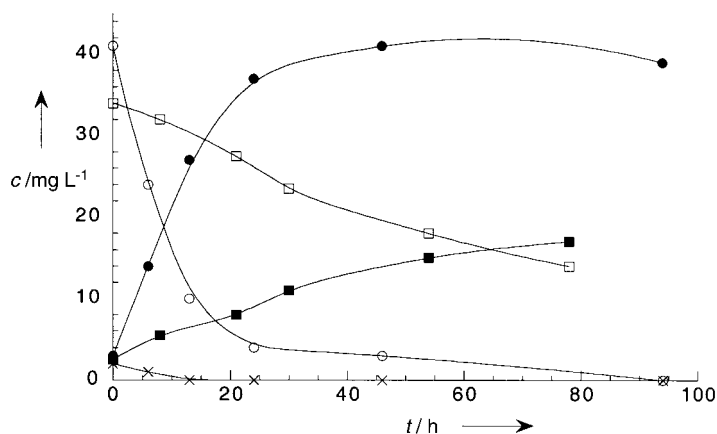
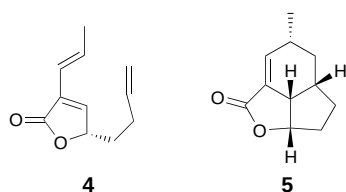


Figure 1. Comparison of the spontaneous cyclization of (–)-**1** (□) to (+)-**2** (■) in abiotic saline at 24 °C with the cyclization of (–)-**1** (○) to (+)-**2** (×) and the formation of (–)-**3** (●) at 24 °C in the presence of active mycelia from *Galiella rufa* (2 g L^{–1} mycelium dry weight) in saline.

saline ($k = 0.00018 \text{ min}^{-1}$) is approximately 8 times lower. When the mycelium was autoclaved prior to the experiment the rate enhancement was lost completely, showing that no unspecific interaction of the mycelium is affecting the cyclization and thus suggesting that the cyclization is catalyzed by a macromolecule with a well-defined structure. In addition, the catalyzed cyclization is highly specific. The



cyclization of the nonnatural enantiomer (+)-pregaliellalactone (**4**) to **5** was not accelerated by the mycelium but proceeded at the same rate as the spontaneous reaction.

The proposed Diels–Alder reaction in the biosynthesis of (–)-galiellalactone (**3**) is different from the two earlier reported biologically catalyzed Diels–Alder reactions. In both previous cases, 1,2-dialkyldecalins were formed from an *E,E,E* triene, the dienophiles were activated by an electron-withdrawing group, and the enzymes catalyzed the formation of diastereomeric products which are not observed from the spontaneous chemical reaction. Clearly, these enzymes stabilize a nonfavored transition state. In the conversion of (–)-**1** to (+)-**2**, in which the dienophile is unactivated, the products of the spontaneous and biologically induced cyclizations are the same. The function of a presumed enzyme must therefore be purely rate enhancing. No attempts were yet made to isolate the factor responsible for the rate enhancement of the cyclization of (–)-**1** to (+)-**2**.

Experimental Section

General: The fungus (*Galiella rufa* A75-86) was grown in a growth medium. EIMS spectra (direct inlet, 70 eV) were recorded with a JEOL SX102 spectrometer, and the NMR spectra (in CDCl₃) with a Bruker

DRX300 spectrometer at 300 MHz (¹H) and at 75 MHz (¹³C), a Bruker DRX400 spectrometer (at 400/100 MHz) and a Bruker ARX500 spectrometer (at 500/125 MHz). All flash chromatography was performed on 60 Å 35–70 µm Matrex silica gel (Grace Amicon). HPLC separation was conducted on a HP1090 series I (column: Merck LiChroCART 125-4, LiChrospher 100, RP-18, 5 µm; linear water–acetonitrile gradient). Thin layer chromatographic analyses were made on Silica Gel 60 F₂₅₄ (Merck) plates and visualized with anisaldehyde/sulphuric acid and heating. Natural (–)-**1**, (+)-**2**, and (–)-**3** were isolated from fermentations of *Galiella rufa* as described previously.^[9]

Cyclization of (–)-**1** to (+)-**2** in toluene: Compound **1** (40 mg, 0.22 mmol) was dissolved in toluene (5 mL) in a sealable tube. The sealed tube was heated at 140 °C for 5 h and then cooled to room temperature. The solvent was removed under reduced pressure and the residue was purified with flash chromatography (hexane/ether 4:1) to afford 32 mg (80%) of **2** as an oil. $[\alpha]_D^{20} = +69.9$ ($c = 0.98$, CHCl₃); ¹H NMR (CDCl₃): $\delta = 0.78$ (1, m), 1.15 (1, m), 1.19 (3, d, $J = 6.8$ Hz), 1.75 (1, m), 1.85 (2, m), 2.10 (1, m), 2.14 (1, m), 2.43 (1, m), 3.05 (1, m), 5.02 (1, m), 6.82 ppm (1, dd, $J_1 = 3.6$, $J_2 = 3.6$ Hz); ¹³C NMR (CDCl₃): $\delta = 20.1$, 31.4, 31.6, 33.7, 34.5, 37.3, 44.2, 83.5, 129.9, 144.7, 170.4 ppm; HRMS (EI) calcd for C₁₁H₁₄O₂ 178.0994, found 178.0995.

Cyclization of (–)-**1** to (+)-**2** in water: Compound (–)-**1** (20 mg, 0.22 mmol) was dissolved in acetonitrile (0.1 mL) and the solution was added to water (110 mL). The solution was stirred at room temperature. Aliquots of 20 mL were removed at regular intervals and extracted with CH₂Cl₂. The organic phase was dried and concentrated, and the relative amounts of **1** and **2** were determined by integration of the peaks in the ¹H NMR spectra.

Cyclization of (–)-**1** to (+)-**2** in LiCl aqueous solution: This experiment was carried out in a 4.86 M LiCl aqueous solution instead of in water; all other parameters were identical to those given above.

Feeding experiments with (–)-**1** and mycelium of *Galiella rufa* strain A75-86: (–)-**1** (40–80 mg) was dissolved in methanol (1 mL) and added to mycelium of *G. rufa* in saline (0.9% NaCl) in a flask as reported previously.^[12] The flask was incubated on a rotary shaker at 120 rpm and 24 °C, and aliquots were removed at regular intervals and analyzed by HPLC with the natural products as reference compounds.

Preparation of (+)-**4**: The synthesis was analogous to that of (–)-**1**,^[12] except for the enantioselective reduction to yield the alcohol (S)-4-hydroxyoct-7-en-2-ynoic acid ethyl ester which was used to prepare **4**: To a round-bottomed flask was added (S)-Alpine-Borane ((S)-B-isopinocampheyl-9-borabicyclo[3.3.1]nonane, 0.5 M in THF; 14.43 mL, 7.21 mmol). THF was removed under reduced pressure. The remaining yellow oil was cooled with an ice bath, and 4-oxooct-7-en-2-ynoic acid ethyl ester (650 mg, 3.6 mmol) was added dropwise. The reaction mixture was stirred overnight and allowed to reach room temperature. The solution was cooled to 0 °C and acetaldehyde (2210 µL, 39.6 mmol in 5.5 mL THF) was added dropwise; then, the solution was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the remaining oil was dissolved in 5.5 mL diethyl ether and cooled to 0 °C. 1,2-diaminoethanol (435 µL, 7.21 mmol) was added dropwise, which led to the precipitation of a thick white solid. The reaction mixture was filtered and the filter washed with several portions of diethyl ether. The filtrate was concentrated. Flash chromatography (CH₂Cl₂/petroleum ether, 50 to 100% CH₂Cl₂) afforded 391 mg (60%) of the alcohol (S)-4-hydroxyoct-7-en-2-ynoic acid ethyl ester. $[\alpha]_D^{20} = +10.5$ ($c = 2.7$, CHCl₃); ¹H NMR (CDCl₃): $\delta = 1.31$ (3, t, $J = 7.1$ Hz), 1.86 (2, m), 2.24 (2, m), 3.11 (1, d, $J = 4.1$ Hz), 4.23 (2, q, $J = 7.1$ Hz), 4.50 (1, q, $J = 6.0$ Hz), 5.01 (1, dq, $J_1 = 10.2$, $J_2 = 1.4$ Hz), 5.08 (1, dq, $J_1 = 17.3$, $J_2 = 1.6$ Hz), 5.80 ppm (1, ddt, $J_1 = 6.7$, $J_2 = 10.3$, $J_3 = 16.9$ Hz); ¹³C NMR (CDCl₃): $\delta = 14.4$, 29.5, 36.2, 61.7, 62.7, 77.0, 88.3, 116.1, 137.5, 154.0 ppm; HRMS (EI) calcd for C₁₀H₁₅O₃ ($M+H$) 183.1021, found 183.1014.

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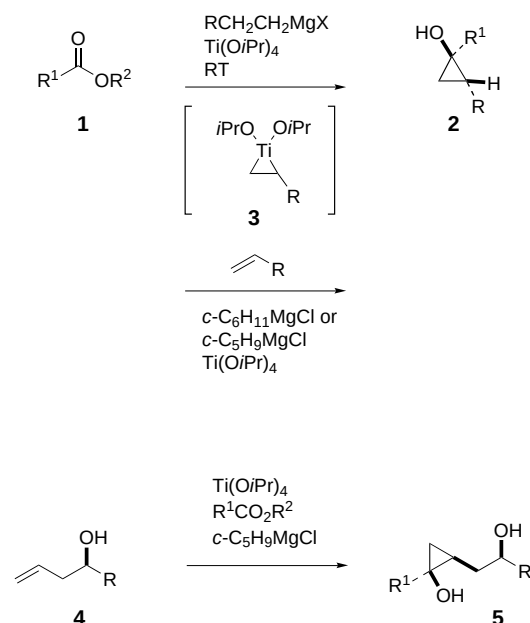
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Diastereoselective Synthesis of *trans*-1,2-Dialkylcyclopropanols by the Kulinkovich Hydroxycyclopropanation of Homoallylic Alcohols**

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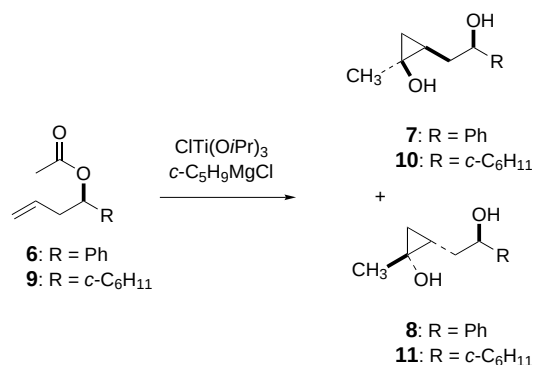
Kulinkovich and co-workers first reported an efficient preparation of cyclopropanols (**1** → **2**), by simple addition of a suitable Grignard reagent to a carboxylic ester, in the presence of titanium tetrakisopropoxide (Scheme 1).^[1] A key intermediate in the Kulinkovich cyclopropanation was presumed to be dialkoxytitanacyclopropane **3**.^[2] An intramolecular process was developed independently by Sato's group and in our laboratories. Subsequently reported was a useful variant of the original Kulinkovich procedure, by facile olefin exchange of the initially formed titanacyclopropane with a terminal olefin, by employing a cyclohexyl or a cyclopentyl Grignard reagent.^[1d, 3, 4] Other studies have included extension of the Kulinkovich cyclopropanation reaction to other



Scheme 1.

acyl derivatives and also applications of the resulting heteroatom-substituted cyclopropanes in organic synthesis.^[2, 5] An enantioselective (70–78% *ee*) synthesis of a *cis*-1,2-dialkylcyclopropanol was also achieved by Corey, who used a TADDOL-derived titanacyclopropane (TADDOL = (*R,R*)- $\alpha,\alpha,\alpha',\alpha'$ -tetraaryl-1,3-dioxolane-4,5-dimethanol),^[6] but its scope and generality remain untested. We report herein a diastereoselective synthesis of *trans*-1,2-dialkylcyclopropanols **5**, which starts with homoallylic alcohols **4**.^[7]

Two different modes of the titanium-mediated hydroxycyclopropanation were available for homoallylic alcohols. One approach entailed an intramolecular cyclopropanation of the esters of homoallylic alcohols (Scheme 2). The related cyclopropanation reaction of but-3-enol esters was established to



Scheme 2.

give *trans*-1,2-dialkylcycloalkanol.^[3a, 4a, 8] As a preliminary study with secondary homoallylic alcohols, an intramolecular cyclopropanation of acetate **6** was first examined by employing an excess of cyclopentylmagnesium chloride in the presence of $\text{Ti}(\text{O}i\text{Pr})_4$ or $\text{ClTi}(\text{O}i\text{Pr})_3$, to obtain cyclopropanols **7** and **8** as a $\approx 1:1$ mixture in 73% yield, along with trace

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