Biosynthetic Diels–Alder Reactions

Chemistry and Biology of Biosynthetic Diels–Alder Reactions

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Nature’s repertoire of biosynthetic transformations has recently been recognized to include the Diels–Alder cycloaddition reaction. Evidence now exists that there are enzymes that mediate the Diels–Alder reaction in secondary metabolic biosynthetic pathways. 2002 marked the 100th anniversary of Alder’s birth and 75 years since the discovery of the Diels–Alder reaction. It would appear that living systems discovered and made use of this ubiquitously useful ring-forming reaction eons ago for the construction of complex natural products. In this Review an overview is given of all of the known classes of natural products (polyketides, isoprenoids, phenylpropanoids, alkaloids) that have been speculated to arise by a biological Diels–Alder reaction.

1. Introduction

The Diels–Alder reaction is a powerful reaction for the formation of carbon–carbon bonds in synthetic organic chemistry which allows facile, stereospecific entry into six-membered ring systems.[1] The structures of various secondary metabolites have led to an array of provocative proposals which suggest that nature might also make use of this valuable reaction.[2] An intriguing aspect of many of these biosynthetic proposals involves the possibility of enzymatic catalysis of the [4+2] cycloaddition, which would accommodate the stereochemistry extant in the respective natural product. Enzymes generally catalyze reactions by stabilizing the structure and charge of the developing transition state. For most reactions subject to this stratagem of catalysis, both the starting substrate and the product differ significantly from the transition state with respect to structure. Both the product and the starting substrate must bind to the enzyme less tightly than the transition-state structure for catalysis to occur. The transition state in the Diels–Alder reaction is highly ordered and closely resembles the structure of the product. In other words, an enzyme that was designed to stabilize the transition-state structure for this reaction would be expected to be inhibited by the product (by tight binding) and turnover (thus, catalysis) would be precluded. Alternatively, the free energy of activation can be lowered by raising the ground-state energy of the reactants. This might be accomplished in the Diels–Alder reaction by the introduction of torsional strain into the dienophile or diene components, but it is difficult to find solid precedent for this strategy in the literature. The prospect of discovering a Diels–Alderase is therefore especially enticing to mechanistic enzymologists, since it could represent a new mechanism of catalysis in nature.

Until recently, the existence of a Diels–Alderase has remained elusive, but catalysis of the Diels–Alder cycloaddition reaction by biomolecules has indeed been realized. Hilvert et al. first reported the catalysis of a Diels–Alder reaction by an antibody in 1989.[3] Monoclonal antibodies were raised against hapten 1 (Scheme 1), which resembles the transition state of the Diels–Alder reaction between tetrachlorothiophene dioxide (3) and N-ethylmaleimide (4). The antibody catalyzed the Diels–Alder reaction by binding the diene 3 and dienophile 4 in a reactive conformation, thus lowering the entropy of activation. The problem of product inhibition was overcome by the extrusion of SO2 from the labile cycloadduct to give a product 5 that did not resemble 1 and, thus, catalyst turnover was not impeded.

Braisted and Schultz used an alternative approach to overcome the difficulty of product inhibition in Diels–Alder catalysis by an antibody (Scheme 1).[4] They used an ethano bridge to lock the cyclohexene ring of the hapten 6 into a conformation resembling the proposed transition state 7 for the Diels–Alder reaction between the acyclic diene 8 and the dienophile 9. The authors argue that the product 10 prefers a twisted chair conformation relative to the rigid boat conformation induced by the bicyclo[2.2.2] hapten 6 that allows for release from the antibody combining site. Subsequent structural elucidation of the complex formed between this catalytic antibody and the hapten revealed the presence of 89 van der Waals interactions and two hydrogen bonds between the antibody and its hapten. These interactions apparently activate the dienophile and control the relative geometries of the bound substrates.[5]

Another type of biomolecule used to catalyze the Diels–Alder reaction is ribonucleic acid (RNA).[6] However, the mechanism of catalysis is radically different from that of catalytic antibodies. Since RNA Diels–Alderase activity is reliant on base specificity and the coordination of a transition metal, the mode of catalysis is more likely akin to Lewis acid catalysis of the Diels–Alder reaction.

The role of protein organization in natural systems and the possible mechanism of catalysis has long been a subject of debate, and was rekindled by the recent characterization of two naturally occurring potential Diels–Alderases.[7,8] The isolation of these enzymes also...
establishes the Diels–Alder reaction as a viable biosynthetic transformation.

This Review is intended to provide an overview of the natural products that have been proposed in the literature to be constructed biosynthetically by a Diels–Alder reaction, both catalyzed and uncatalyzed. Where available, the biosynthetic studies pertaining to these substances to prove these questions are summarized. Although there are countless structures that can formally be envisioned to arise by a [4+2] cycloaddition, this Review is limited to those natural products that have been described in the literature as putative Diels–Alder cycloadducts. The Review is organized into classes of compounds based on their biosynthetic derivations: polyketides (acetate), isoprenoids (mevalonate), phenylpropanoids (shikimic acid), and alkaloids (amino acids). In many cases, this segregation is superficial, since many compounds are often of mixed biosynthetic origins (for example, cytchalasans, pycnidione, and brevianamides).

2. Polyketides

Since polyketides are derived from acetate, these compounds are particularly well-suited for isotopic labeling studies. For example, isotopically labeled precursors, such as $^{13}$C-acetate, are readily accessible, comparatively cheap, and are often readily incorporated into the corresponding secondary metabolite in feeding experiments. Thus, it is hardly surprising that a large portion of the experimental evidence for the Diels–Alder reaction in nature has been obtained for this class of compounds. In fact, both lovastatin and macrophomic acid, for which reported Diels–Alderase enzymes have been isolated,[7,8] are classified as polyketides.
2.1. Decalin Polyketides

2.1.1. Lovastatin (Mevinolin)

Lovastatin (11), also known as mevinolin, has received significant attention in the literature, since it is a potent inhibitor of cholesterol biosynthesis in humans and has become a clinically useful and very successful drug. Feeding experiments on the producing fungal strain Aspergillus terreus (ATCC 20542) with [1-13C], [2-13C], [1,2-13C2], [1-13C,1-18O2]-, [1-13C,2-2H3]-, and [2-13C,2-2H3]acetates established that lovastatin is comprised of a C18 unit and a C4 unit constructed through the head-to-tail attachment of acetate (Scheme 2).[9]

Interestingly, of the five oxygen atoms in lovastatin, only the oxygen atom attached at C11 could definitively be assigned as a derivative of acetate. In addition, it was discovered that the two methyl groups at C2 and C6 are derived from S-adenosylmethionine (SAM), as shown by feeding experiments with [13CH3]methionine.

Re-examination of the origin of the oxygen atoms in lovastatin (11) was made possible by the subculture selection of a new strain of Aspergillus terreus (MF 4845), which increased the production of 11 to 200 mg per litre of culture.[10] Fermentation of Aspergillus terreus in the presence of an 18O2-enriched atmosphere showed the oxygen atom at C8 was derived from molecular oxygen. A separate feeding experiment with [1-13C,1-18O2]acetate indicated the oxygen atoms at C1', C11, C13, and C15 are derived from acetate. The results of these feeding experiments as well as the previous experiments led to the biosynthetic hypothesis outlined in Scheme 2.[9,13] Vederas and co-workers speculated that the enzymes involved in the biosynthesis of lovastatin (11) are similar to those involved in the biosynthesis of fatty acids.[9] They proposed that condensation of acetate units (from malonate) could produce a triene 12 that would undergo an endo-selective Diels–Alder cyclization to the decalin 13.

The first test of this hypothesis was a synthesis of the analogue 14 through laboratory Diels–Alder cyclizations of the thioester 15a, ethyl ester 15b, and acid 15c both thermally and with a Lewis acid catalyst (Scheme 3).[11] There was a 1:1 ratio of the endo(14c):exo(14d) products in the thermal cyclization, presumably through a chairlike transition state with the methyl side chain disposed in a pseudo-equatorial manner. However, no endo product 14a corresponding to the stereochemistry of 11 was observed (with a pseudo-axial methyl side chain in the transition state). The Lewis acid catalyzed Diels–Alder reaction gave the same two products as the thermal reaction but in a 9:1 endo:exo ratio for 15b and a 19:1 ratio for 15a. The absence of product 14a in the laboratory cyclization suggests that the Diels–Alder cyclization in the biogenesis of 11 could be enzymatic.

Feeding experiments were performed under a variety of conditions on Aspergillus terreus (MF4845) with 15a doubly labeled with 13C at C2 and C11 to test for Diels–Alder activity in vivo (Scheme 4).[11] This doubly labeled precursor readily degrades by β-oxidation to smaller building blocks (for example, acetate). Incorporation of the intact precursor would lead to adjacent 13C labels, which would be perceived as carbon–carbon coupling in the 13C NMR spectrum. However, feeding experiments with [2,11-13C]15a did not reveal detectable 13C–13C coupling in the 13C NMR spectra. Appa-

Scheme 2. Origin of the carbon atoms of lovastatin (11) and proposed biogenesis.[9,10]

Scheme 3. Synthesis of the decalin 14 though an in vitro Diels–Alder cyclization of triene 15. Thermally: toluene, 160°C, 4 days; Lewis acid catalyzed: 0.9 equiv EtAlCl2, RT, 3 h.[11]
Currently, 15a was catabolized before it could undergo cycloaddition.

A complete outline of the proposed biosynthesis of 11 including the role of the lovB and lovC genes is shown in Scheme 5. Vederas, Hutchinson, and co-workers demonstrated that dihydromonacolin L (25), an established intermediate in the biosynthesis of lovastatin (11), was formed in a heterologous host, Aspergillus nidulans, containing the lovB and lovC genes from Aspergillus terreus. In addition, expression of the lovB protein (lovastatin nonaketide synthase, LNKS) in the absence of lovC protein led to truncated pyrones because of the inefficient enoyl reduction at the tetraketide stage. These results were interpreted as supporting the notion of catalytic Diels–Alder activity for LNKS.

The enzymatic activity of LNKS was tested on 15a, the analogue of the proposed cycloaddition precursor. The N-acetylcysteamine (NAC) ester 15a (Scheme 6) was added to an aqueous buffered solution containing pure homogeneous LNKS protein. The endo-Diels–Alder product 14a, which had the same stereochemistry observed in 11, was obtained along with the non-enzymatic products 14c and 14d (14a:14c:14d = 1:15:15). The cis-fused exo-product 14b was not observed under any conditions. When 15a was added to thermally denatured LNKS, adducts 14c and 14d were formed, but 14a was not detected. Cycloadducts 14c and 14d result from a transition state with the C6 methyl group in a sterically favored pseudo-equatorial arrangement. However, the transition state leading to 14a requires a more crowded pseudo-axial disposition of the methyl group at C6. Thus, it seems the function of LNKS is to bind the substrate in a conformation that resembles the endo transition state that leads to 14a (analogous to the mode of action of catalytic antibodies). In addition, hydrogen bonding of the carbonyl oxygen atom within the active site of LNKS would make the dienophile more electron deficient, thus resembling Lewis acid catalysis of laboratory Diels–Alder reactions. Since the product 14a was not obtained in the presence of denatured LNKS, the asymmetric induction of the Diels–Alder reaction cannot be caused by nonspecific binding of a chiral protein. Therefore, LNKS represents the first naturally occurring Diels–Alderase enzyme to be purified to homogeneity.


Scheme 5. Proposed biosynthetic pathway for lovastatin (11). The boxed region shows reactions catalyzed with LNKS and the lovC protein. The domains for the LNKS and the lovC protein were assigned from sequence homology to other polyketide synthase (PKS) proteins. KR = keto reductase, DH = dehydratase, MeT = methyltransferase, ER = enoyl reductase, KS = [β-ketoacyl synthase, ACP = acyl carrier protein, AT/MT = acetyl/malonyltransferase.
2.1.2. Solanapyrones

Another decalin polyeatomite thought to arise through a [4+2] cycloaddition is solanapyrone, a phytotoxin produced by the pathogenic fungus, *Alternaria solani*.[15] A series of feeding experiments with singly and multiply labeled acetate and [1-13C,1-18O]acetate excluded the possibility that the polyketide is derived by oxidative scission of a longer precursor and proved that the polyketide is responsible for producing the optically pure [31] conversion of the [2H7]-acetate in the natural system, this was the first indication that the reaction might be enzyme-mediated.

Evidence for the biosynthetic Diels–Alder reaction in the biosynthesis of solanapyrone was obtained when the achiral deuterated trienes 26 and 31 were incorporated in vivo into 26 and 32,[16,19] Incorporation of the precursor 30 indicated loss of deuterium at C17. The ratio of the integration for the signals of deuterium at C17 to deuterium at C18 in the H NMR spectrum changed from 2:3 in 30 to a ratio of 1:5:1 for 26 and 1:5:1 for 32. Observation of the same deuteration ratio for 26 and 32 indicates that 26 is reduced to 32 and that the two triene precursors 30 and 31 are oxidized to the same intermediate, presumably the C17 aldehyde proslanapyrone II (33). Feeding experiments could not be performed with 33 because it was so reactive and underwent spontaneous endo cyclization in aqueous conditions.

The reactivity of the aldehyde 33 makes it a likely candidate as a direct substrate for the Diels–Alder cyclization (Scheme 8). Incorporation of the [3H]-31 with an essentially unchanged deuteration ratio demonstrated that 30 was not incorporated into 26 or 32, which indicates that the Diels–Alder reaction probably occurs after oxidation.

Enzymatic activity was found in a cell-free extract of *Alternaria solani*, which catalyzed the conversion of 33 into solanapyrones A (26) and B (27) in 25% yield with a ratio of the *exo* to *endo* cycloadduct of 53:47.[21] A control experiment with denatured enzyme provided a 3:97 ratio of the *exo* to *endo* cycloadducts with only 10% consumption of starting material. The observed stereoselectivity in the cell-free extract was interpreted as being indicative of enzymatic activity.

Conversion of 31 with the crude enzyme preparation was accomplished in 25% yield (19% 26 and 27, 6% 33) with an *exo*/*endo*-cycloaddition ratio of 85:15 and an optical purity (for 26) of 99% ee. The optical purity of 26 produced from 33 was 92% ee. Since this value is lower than that obtained from 31, it seems that a single enzyme catalyzing the oxidation and cycloaddition is responsible for producing the optically pure solanapyrones found in the natural system. Further proof of

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**Scheme 6.** Synthesis of 14 through in vitro enzymatic Diels–Alder cyclization.[17]
this sequence of events was obtained when the enzymatic cycloaddition reaction of 31 was suppressed in the absence of oxygen (argon atmosphere).

2.1.3. Nargenicin

Nargenicin (37), a polyketide antibiotic isolated from *Nocardia argentinensis*, contains a macrocyclic lactone fused to a cis-octahydronaphthaline (octalin) ring system that is derived biosynthetically from five acetate and four propionate units. Feeding experiments with [1-18O2,1-13C]acetate and [1-18O2,1-13C]propionate indicated that the oxygen atoms at C1 and C11 were derived from acetate while the oxygen atoms at C9 and C17 were derived from propionate (Scheme 9, inset). In accord with these results, incubation of *N. argentinensis* with 13C-labeled acetate and propionate in an 18O2-enriched atmosphere indicated that the two ether oxygen atoms at C2 and at C13 and the hydroxy group at C18 are derived from molecular oxygen.

Since the oxygen atom at C13 is not derived from propionate, this implies that the C4–C13 bond is not formed by an aldol-type condensation. Instead, a Diels–Alder cyclization was invoked through the intermediacy of the triene 38 (Scheme 9). By the incorporation of the 13C- and/or 2H-labeled NAC esters of the precursors 40, 41, 42, and 44 (Scheme 9), Cane et al. demonstrated that the stereochemistry and level of oxidation are set prior to chain elongation. The incorporation of [13C]-44 also further supports the notion that the cis-octalin ring system is generated through a Diels–Alder cycloaddition.

2.1.4. Betaenone B

Betaenone B (45) is a phytotoxin from *Phoma betae* (a fungus) that causes a leaf spot disease on sugar beet. Feeding experiments with [1-13C,1-18O2]acetate indicated that only the oxygen atom at C16 of 45 is derived from acetate (Scheme 10). The absence of an 18O-induced isotopic shift in the signal corresponding to C18 could indicate that the oxygen atom is derived from molecular oxygen or it could indicate a “washing out” of the label by exchange with water. When a P450 inhibitor, ancymidol (46), was added to cultures of *P. betae*, the production of betaenone B (45) was suppressed in proportion to the amount of inhibitor added. In addition, a new deoxygenated metabolite, probetaenone I (47), was isolated which was proposed to be a biosynthetic precursor of 45 through the intramolecular Diels–Alder reaction of the projected intermediate 48.

Probetaenone I (47) was later proven to be a precursor to 45 (Scheme 10). Separate feeding experiments of *Phoma betae* with [1-14C]acetate, [1,2-13C2]acetate, and [S-13CH3]methionine in the presence of the P450 inhibitor SD-3307D (49) provided labeled 47. Subsequent feeding experiments with each labeled probetaenone I (47) displayed incorporation into 45 (6.02% incorporation from [1-14C]acetate, 19.1% enrichment from [1,2-13C2]acetate, and 9.6% enrichment from [S-13CH3]methionine). Synthesis of 47 through an intramolecular Diels–Alder reaction confirmed the structure and provided credence for the proposed biosynthetic pathway.

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Scheme 8. Incorporation of deuterated trienes into solanapyrones A (26) and D (27). The thioacetal of 27 is the major product (2:1) in the laboratory synthesis.

Scheme 9. Incorporation of deuterated trienes into solanapyrones A (26) and D (27). The thioacetal of 27 is the major product (2:1) in the laboratory synthesis.
2.2. Macrocyclic Polyketides

2.2.1. Cytochalasans

The cytochalasans are a large family of macrocyclic polyketides that possess cytostatic activity. To date, approximately 60 natural products belonging to this class of mycotoxins have been isolated. Structurally, the cytochalasans are characterized by a highly substituted perhydroisoindole group fused to a macrocyclic ring to give the four basic skeletal structures A–D. The majority of the macrocycles are carbocyclic, but the macrocycle can also be a lactone or cyclic carbonate.
Feeding experiments have established acetate, propionate, methionine, phenylalanine, and tryptophan as biosynthetic precursors to the cytochalasans. The perhydroisoindole group of cytochalasin A (50) and B (51) is thought to arise through an endo-selective intramolecular Diels–Alder reaction (Scheme 11). Incorporation of deoxaphomin (57) into 51 indicates that oxidation to the macrolide occurs after the putative Diels–Alder cyclization and implies that there is a common biosynthetic pathway for the cytochalasans.

Indirect evidence for the Diels–Alder-mediated biosynthesis of the cytochalasins was obtained by feeding and inhibition experiments with Chaetomium subaffine, which produces chaetoglobosin A (58, Scheme 12). A feeding experiment with [1-13C,2-2H3]acetate showed retention of the deuterium labels at C11, C8, and C14. Retention of deuterium at C8 and C14 precludes formation of the perhydroisoindole and macrocycle through a proposed formation of a carbon–carbon bond in which a carbonyl group is located at C14. A feeding experiment with [1-13C,1-18O2]acetate established that the oxygen atoms at C1 and C23 originate from the acetate, while incubation in an 18O2-enriched atmosphere displayed an upfield shift of the C6, C7, and C20 signals in the NMR spectrum. An inhibition experiment with the cytochrome P450 inhibitor metapyrone led to the formation of the metabolites 59–62 (with 59 as the major product). These results led to the biosynthetic proposal for the formation of 58 outlined in Scheme 12. An intramolecular Diels–Alder reaction of the putative hexaene 63 would provide 59, which could then undergo a stepwise oxidation to provide 58.

The possibility for enzymatic involvement in the proposed Diels–Alder cyclization of the cytochalasans was evidenced by the retro-Diels–Alder reaction of 59 (Scheme 13). Instead of forming the expected triene 63, pyrolysis (180°C, sealed tube) of 59 produced equal amounts of starting material and the diastereomer 65. The lack of stereoselectivity in the thermal Diels–Alder reaction supports the hypothesis that an enzyme preorganizes the substrate conformation to favor the endo-transition state in the biological system, which results in exclusive formation of 59.

2.2.2. Cochleamycins

The polyketide origin of cochleamycins A (66) and B (67), produced by Streptomyces sp. strain DT136, was determined from feeding experiments with [1-13C]acetate, [2-13C]acetate,
Based on these results, the biosynthesis shown in Scheme 15 was proposed. Oxidation of the allylic methyl group in the proposed intermediate 70 followed by an intramolecular Diels–Alder reaction and aldol condensation could lead to the formation of 66. Formation of 67 is thought to arise from reductive transannular cyclization at the C4- and C16-positions of 66 accompanied by elimination of the hydroxy group at C16. The desired stereochemistry for the intramolecular Diels–Alder reaction at the AB- and BC-ring junctures can be obtained by endo addition of the trans-olefin at the C6-position to the 11-trans-13-cis-diene, or by the exo addition of the trans olefin to the 11-cis,13-trans-diene (Scheme 15, inset).

2.2.3. Ikarugamycin

Ikarugamycin (75) is a member of a small family of macrocyclic antibiotics produced by Streptomyces phaeochromogenes var. ikaruganensis Sakai, which possess an unusual perhydro-as-indacene ring system. Other members of this family include lepicidin A (76, A83543A) and capsimycin (77). The structure and stereochemistry of ikarugamycin were determined by Ito and Hirata in 1972. They proposed that ikarugamycin was biosynthesized from two hexaacetate units 78 and l-ornithine, and that the decahydropyridacene skeleton

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\text{Scheme 13. Retro-Diels–Alder reaction of the chaetoglobosin A precursor 59.}^{[35]}
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\text{Scheme 14. Incorporation of acetate and propionic acid into cochleamycin A (66) and B (67).}^{[37]}
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\text{Scheme 15. Proposed biosynthetic pathway of cochleamycins A (66) and B (67).}^{[37]}
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Diels–Alder reaction via a pentaene intermediate such as that the biosynthesis of Perhydroindane Polyketides family of natural products have not yet appeared.

Scheme 17. Biomimetic synthesis of the perhydro-αs-indacene ring system of 75. KHMDS = potassium hexamethyldisilazide, TBS = tributylsilyl, Tf = trifluoromethanesulfonyl, HMPA = hexamethyl phosphoramide.

2.3. Perhydroindane Polyketides

Indanomycin (83, X14547A) is a member of a small family of polyketides produced by Streptomyces antibioticus which contain a perhydroindane skeleton. Other members of this family include the antibiotic A83099A (84), which is produced by Streptomyces setonii; the marine natural product pulo’upone (85), which is produced by the mollusk Philinopsis speciosa; and stawamycin (86), produced by a Streptomyces sp. Tularik 8349. Roush et al. hypothesized that the biosynthesis of 83 might involve an intramolecular Diels–Alder reaction via a pentaene intermediate such as 89. Based on this hypothesis, the total synthesis of indanomycin was completed by using an intramolecular [4+2] cycloaddition of 89 as the key step (Scheme 18). The isomerically pure product was obtained in 51% yield with 5% of a mixture of cis-fused products and 5% of the C10,11-Z isomer.

2.4. Other Polyketides

2.4.1. Endiandric Acids

The endiandric acids are isolates from the leaves of the Australian plant Endiandra introrsa (Lauraceae). Since endiandric acid A (90) and B (91) occur together with endiandric acid C (92), and as these compounds are isolated in racemic form, Bandaranayake et al. postulated there was a unified biogenesis involving a series of electrocyclizations from an achiral precursor (Scheme 19). They proposed that a polyketide of type 93 might lead to a phenylpolyene acid with a central conjugated tetraene unit. An 8ₐ conrotatory electrocyclication of the all-cis tetraene 94a or the trans,cis,cis,trans isomer 94b, followed by a 6ₐ disrotatory electrocyclization and finally an intramolecular Diels–Alder (4τs+2τs) cycloaddition would provide 90 and 91 or 92, respectively.

Nicolaou et al. explored the feasibility of the proposed pathway though a biomimetic synthesis. Stepwise stereo-controlled syntheses of endiandric acids A–D (90–92, 101) were first completed to determine if the proposed sequence of events was viable. Next, they completed a one-pot electrocyclic cascade reaction. Hydrogenation of the acyclic precursor 99 (Scheme 20) with Lindlar’s catalyst provided the methyl esters of endiandric acids E and D (100 and 101), while brief heating of the reaction provided the methyl ester (102) of endiandric acid A. An analogue of 99 with an elongated chain was used to synthesize 91 and 92 along with
the unnatural endiandric acids G–F. These syntheses validate the biosynthetic hypothesis of Bandaranayake et al. and indicate that these electrocyclic reactions are not enzymatically catalyzed. In addition, the syntheses of the unnatural endiandric acids E–G may help in the identification of these compounds in the natural system.

2.4.2. Bisorbicillinoids

The bisorbicillinoids (103–109) are a growing family of mycotoxins that are proposed to arise from a common biosynthetic precursor, sorbicillin (110, Scheme 21). The 2,3′-dihydro derivative of sorbicillinol (104), the first member of the bisvertinoquinol (103) class of compounds to be isolated, was postulated to be a Diels–Alder adduct of two different quinols derived from the co-metabolites (110) and 2,3′-dihydroisorbicillin through enantioselective oxidation of CS. This route was postulated because the structure of 103 is consistent with a spontaneous endo-selective Diels–Alder reaction. However, the variations in the sorbyl and dehydrosorbyl side chains mean that four Diels–Alder adducts are possible. Only one optically active bisvertinoquinol-type product was observed in the cultures, which

![Scheme 18](image)

Scheme 18. Biomimetic total synthesis of indanomycin (83).[47] The inset shows the structures of related natural products A83099A (84),[44] pulo’upone (85),[45] and stawamycin (86).[46]

![Scheme 19](image)

Scheme 19. Proposed biosynthesis of endiandric acids A–C (90–92).[39]

![Scheme 20](image)

Scheme 20. Biomimetic synthesis of the endiandric acids.[31]
suggests that chain differentiation occurs after the Diels–Alder reaction and that 103 is not an artifact of isolation.

A similar biosynthetic Diels–Alder proposal has been made by Abe, Murata, and Hirota for bisorbicillinol (104), which could form bisorbutenolide (105) through an anionic cascade reaction. [53] Sorbiquinol (106) has also been postulated to arise from a [4+2] cycloaddition. However, for 106, the Diels–Alder reaction would occur between the C2’–C3’ double bond of the sorbicillin (110) side chain as the dienophile and enantioselectively oxidized sorbicillin as the diene. [26] Alternatively, the biosyntheses of sorbicillinolide (107) and trichodimerol (109) can be rationalized as products of an oxidation-Michael-ketalization cascade. [54]

Scheme 21. Biomimetic total syntheses of sorbicillinol mycotoxins from the same precursor 110. [55]

In support of the proposed biosynthetic pathways, two research groups independently and concomitantly completed the biomimetic total syntheses of sorbicillinol (104) and trichodimerol (109, Scheme 22). [55] Nicolaou et al. reported that basic or acidic hydrolysis of the acetoxy functionality of 111 provided the quinols 112a and 112b which spontaneously formed the Diels–Alder cycloadduct 104 (path A in Scheme 22). [55a,b] Four stereogenic centers were created in the Diels–Alder reaction with complete regio- and diastereosecontrol. Additionally, the quinol intermediate 112b has phoenolpyruvate (115) as the C₃ unit. In practice, incubation of a cell-free extract of M. commelinae with 114 and 115 led to the enzymatic formation of 113. [58]

The proposed biosynthetic pathway for macrophoric acid entails an inverse-electron-demand Diels–Alder reaction of 114 and the diene 115. The intermediate 116 is transformed to 113 by successive retro-Diels–Alder reaction and syn elimination of phosphoric acid. To test this hypothesis, an analogue 117 of the putative bicyclic intermediate 116 was synthesized and incubated with the cell-free extract of

2.4.3. Macrophomic Acid

Macrophomic acid (113) is a fungal metabolite isolated from Macrophoma commelinae. Sakurai et al. established that macrophoric acid is derived from an unidentified C₃ unit and the 2-pyrone 114 with loss of CO₂ and an unidentified C₄ unit. [57] Subsequent work on the biosynthesis of macrophoric acid revealed incorporation of [1-13C]l-alanine, [1-13C]l-serine, [U-13C]glycerol, (1R,2S)-[1-2H]glycerol, and (1R,2R)-[1-2H]glycerol. [58] Based on these experiments, Oikawa et al. proposed the biosynthetic pathway outlined in Scheme 23 with phos-
M. commelinae, 114, and 115. The analogue 117 inhibited the formation of 113 (IC₅₀ value 200 µM).[[58]]

A recent re-examination of the origin of the C₃ unit led to the discovery that oxaloacetate (118) is a more efficient and direct precursor to 113. The Mg²⁺-dependent enzyme macrophomate synthase was isolated and purified by using oxaloacetate as the sole substrate for the C3 unit.[[59,60]] This single enzyme, a homodimer of a 36-kDa protein, was found to catalyze a five-step transformation involving two decarboxylations, two C–C bond-forming reactions, and a dehydrogenation.[[6]]

Oikawa and co-workers speculate that the C₃ unit might still be an enol pyruvate (119), the product of oxaloacetate decarboxylation. To test this, macrophomate synthase was incubated with 118 in the absence of 114 in a lactate dehydrogenase coupled assay.[[8]] Rapid formation of pyruvate was observed. However, in a competition experiment, 114 was found to inhibit the conversion of 118 into pyruvate. This observation indicates that the enzymatic product of oxaloacetate decarboxylation is not hydrolyzed and undergoes further reaction with 114 (Scheme 24).

Incubation of macrophomate synthase with oxaloacetate (118) and 2-pyrones lacking a C₄ substituent, such as methyl coumalate (120), result in the formation of aberrant bicyclic compounds such as 123 and 124 (Scheme 25).[[8,61]] The location of the double bond and absence of an oxygen functionality at C₅ suggests that the proposed intermediate 121 undergoes allylic rearrangement and subsequent re-lactonization. The aberrant cycloadduct 123 may be formed instead of the

Scheme 23. Original biosynthetic proposal for macrophomic acid (113).[58] 3-PG = glycerin-3-phosphate.

Scheme 24. Revised proposal for the biosynthesis of 113.[8]

Scheme 25. Proposed mechanism for the formation of side products by a cycloaddition catalyzed by macrophomate synthase.[8]
benzoate because the lack of a C4 substituent causes improper interaction between the catalytic residue and the elimination groups or because the C4 substituent interrupts attack of the carboxylate group on the carbocation. In either case, the driving force for the rearrangement is probably the release of steric strain of 121. Deuterium labeling experiments revealed the pro-R position of adduct 124 is retained, which indicates that the first decarboxylation step provides the Z enolate.[8] The stereochemistry of the decarboxylation reaction is consistent with the known enzyme, phosphoenolpyruvate carboxylase.

Two possible routes, a stepwise Michael–aldol reaction or a concerted Diels–Alder reaction, can account for C–C bond formation by macrophomate synthase (Scheme 24).[8] In the Michael–aldol reaction, attack of the enolate on 114 would provide the first C–C bond and stabilize the negative charge on the 2-pyrene. Subsequently, the enolate could attack the newly formed carbonyl group to afford the bicyclic intermediate 116. However, an intermediate in which only a single C–C bond has been formed, such as 125, has not been observed for reactions catalyzed by macrophomate synthase. In the second case, a Diels–Alder cyclization may resemble an antibody-catalyzed Diels–Alder reaction. The bicyclic intermediate 116 in the macrophomate synthase catalyzed reaction could be stabilized by the groups used for recognition of the enolate and 114.

Support for the Diels–Alder proposal was proffered from a known example of a [4+2] cycloaddition of a 2-pyrene and an equivalent of pyruvate enolate.[62] Oikawa and co-workers interpreted the high stereospecificity observed in aberrant cyclization products from the macrophomate synthase catalyzed cyclization as being consistent with a concerted mechanism. However, since the “normal” reaction products from the synthase are achiral, this is highly speculative. Nevertheless, more information will be needed to determine if the C–C bond-forming reactions of macrophomate synthase indeed arise from a concerted Diels–Alder reaction that is enzyme-mediated.

3. Isoprenoids

3.1. Derivatives of Myrcene and trans-β-Ocimene

3.1.1. Perovskone

The terpenes myrcene and trans-β-ocimene are often utilized as dienes in the construction of Diels–Alder-derived natural products. Perovskone (126) is a triterpene isolated from Perovskia abrotanoides. Initially, it was thought that 126 was constructed from an icetexone precursor 127 and geranyl pyrophosphate (Scheme 26).[63] A [4+2] cycloaddition route from 128 and trans-β-ocimene 129 was later proposed and this concept was used to complete a biomimetic total synthesis (Scheme 27).[64]

3.1.2. Heliocides

Heliocides H₁ (130), H₄ (131), B₁ (132), and B₄ (133) are proposed to be derived from trans-β-ocimene (129) and hemigossypolone (134) or its methyl ether derivative (135). Heliocides B₁ (132) and B₄ (133) were synthesized in a 3:1 ratio by a biomimetic [4+2] cycloaddition; this is the same ratio observed in the isolation of the natural products (Scheme 28).[65] While incorporation studies have not been reported, the biomimetic synthesis shown in Scheme 28 provides indirect, provocative evidence for the postulated biosynthesis.
3.1.3. **Eudesmanolides**

The eudesmanolides 136 and 137 were isolated from the aerial parts of *Artemisia herba-alba*.\[66\] They are formally derived through an inverse-electron-demand Diels–Alder reaction between myrcene (138) as the dienophile and 139 as the dieneone. The synthesis of 136 and 137 was accomplished in a 1:1 ratio by heating 138 and 139 to 100°C (Scheme 29). Since the conditions required for the synthesis of 136 and 137 are so harsh, it is unlikely that they are artifacts of isolation.

![Scheme 29. Biomimetic synthesis of eudesmanolide adducts 136 and 137.](image)

3.2. **α-exo-Methylene-γ-lactones**

3.2.1. **Plagiospirolides**

GC-EIMS analysis was carried out on plagiospirolide A (140) to enable the structures of the spiroterpenoids isolated from the Panamanian liverwort *Plagiochila moritziana* to be determined. Diplophyllolide (141) and fusicoccadiene (142) were detected, possibly resulting from a retro-Diels–Alder reaction, and both substances were isolated from extracts of *Plagiochila moritziana*.\[67\] Further isolations of *Plagiochila moritziana* provided plagiospirolide E (143). Again, GC-EIMS provided potential retro-Diels–Alder products—diplophylline (144) isolated from *Plagiochila moritziana* and the diene (145, Scheme 30).\[68\]

Since a synthetic Diels–Alder route to the related triterpenes from *Helenium autumnale* required harsh conditions and gave low yields of a mixture of isomers,\[69\] it is unlikely that 140 is an artifact of isolation. In addition, since no other diastereomers of 140 were found in *P. moritziana* cultures, it is possible that the putative biosynthetic Diels–Alder reaction is enzymatic.

![Scheme 30. Retro-Diels–Alder fragmentation of plagiospirolide A (140) and plagiospirolide E (143).](image)

3.2.2. **Xanthipungliolide, Pungiolide, and Others**

Besides the plagiospirolides, there are a number of putative Diels–Alder adducts derived from α-exo-methylene-γ-lactones. The species *Xanthium pungens* produces both xanthipungolide (146) and pungiolide (147).\[70\] Both substances were proposed to be biosynthetic derivatives of xanthanolide 148 (Scheme 31). It was proposed that an electrocyclic reaction of 148 forms 149 which is then followed by an intramolecular Diels–Alder reaction in the biogenesis of 146. This proposal was supported by the synthesis of 146 from 148, accomplished by irradiation of 148 in ethanol. The biosynthesis of the dimer 147 is thought to arise from an intermolecular Diels–Alder reaction of 148 followed by an oxidation.

Mexicanin F (150), from *Helenium mexicanin*, is thought to arise from the co-metabolite mexicanin E (151).\[71\] Heating the dimeric sesquiterpene lactone absinthin (152) gives the
monomer artabisin (153).\textsuperscript{[72]} From its fragmentation pattern in the CI mass spectrum, the biogenesis of biennin C (154) was proposed to occur from an intermolecular cycloaddition of the monomers 155 and 156.\textsuperscript{[73]} Ornativolide A (157)\textsuperscript{[74]} and fruticolide (158)\textsuperscript{[75]} could also be [4+2] cycloadducts derived from \(\alpha\)-exo-methylene-\(\gamma\)-lactones (Scheme 32).

\begin{align*}
\text{150, mexicanin F} & \rightarrow \text{151, mexicanin E} \\
\text{152, absinthin} & \rightarrow \text{153, artabisin} \\
\text{154, biennin C} & \rightarrow \text{155} \\
\text{156} & \rightarrow \text{157, ornativolide} \quad \text{158, fruticolide}
\end{align*}

\textbf{Scheme 32.} Diels-Alder cycloadducts derived from \(\alpha\)-exo-methylene-\(\gamma\)-lactones.\textsuperscript{[71–75]}

### 3.3. Homodimer Terpenoids

#### 3.3.1. Torreyanic Acid

Torreyan acid (159) is a cytotoxin isolated from the endophytic fungus Pestalotiopsis microspora.\textsuperscript{[76]} This substance possesses an unusual dimeric quinone structure that was postulated to arise from a Diels–Alder cycloaddition of two diastereomeric monomers. A proposed biosynthetic pathway might involve the following: a) electrocyclic ring closure of achiral 160 to form racemic 161; b) enzymatic oxidation to generate the diastereomers 162a and 162b; and c) a [4+2] cycloaddition to produce 159 (Scheme 33). A biomimetic total synthesis of 159 was recently completed, which employed the [4+2] dimerization of diastereomeric monomers.\textsuperscript{[77]}

\begin{align*}
\text{159, torreyanic acid} & \rightarrow \text{160} \\
\text{161} & \rightarrow \text{162a} \quad \text{162b} \\
\text{163, longithorone} & \rightarrow \text{164, shizukaol A} \\
\text{165, cyclodione} & \rightarrow \text{166, maytenone}
\end{align*}

\textbf{Scheme 33.} Proposed biosynthesis of torreyanic acid (159).\textsuperscript{[76]} \(R = \text{CH}_2\text{CH} = \text{C} (\text{CH}_3) \text{COOH}, R' = \text{C}_6\text{H}_{11}\).

### 3.3.2. Longithorone and Other Homodimer Terpenoids

There are a number of examples of terpenoid homodimers that might arise through a [4+2] cycloaddition. A recent example of a Diels–Alder-cyclized quinone dimer is longithorone (163), isolated from a marine tunicate.\textsuperscript{[78]} Other examples include shizukaol A (164),\textsuperscript{[79]} cyclodione (165),\textsuperscript{[80]} and maytenone (166; Scheme 34).\textsuperscript{[81]}

\begin{align*}
\text{163, longithorone} & \rightarrow \text{164, shizukaol A} \\
\text{165, cyclodione} & \rightarrow \text{166, maytenone}
\end{align*}

\textbf{Scheme 34.} Homodimeric terpenes as possible Diels–Alder cycloadducts.\textsuperscript{[78–81]}

#### 3.3.3. Culantraramine

Caution needs to be taken when considering the biosynthesis of these dimers; for example, culantraramine (167) could be considered as a natural Diels–Alder cycloadduct.\textsuperscript{[82]} However, when the proposed precursor 168 was allowed to...
stand in xylene at room temperature for 10 days, the cyclo-
adducts 169 and 170 were obtained, not the natural product
167 (Scheme 35). On the other hand, when 171 was treated
with acid, the product 167 was formed at room temperature
within 30 minutes. Thus, it seems that the biosynthesis of 167
does not occur through a “true” Diels–Alder cyclization, but
perhaps through a nonsynchronous cation–diene [4
+ 2] cyclo-
addition.

3.4. Other Isoprenoids

3.4.1. Ircinianin and Wistarin

Ircinianin (172) is a sesterterpene isolated from the
marine sponge Ircinia wistarii. It was postulated to arise
from a [4+2] cycloaddition of the linear tetraene (173).[83]
Both the racemate and the (−) isomer of 172 have been
synthesized utilizing this approach.[84] Wistarin (175) is a
tetracyclic isomer of tricyclic 172. Interestingly, both the (+)
and (−) isomers of 175 have been isolated, but only one
enantiomer of 172 has been isolated (Scheme 36).[85] This
observation could be considered as evidence that the
formation of 175 is mediated by enzyme catalysis.

3.4.2. Mirosterol

Mirosterol (176) is an estrogenic phenol isolated from
the Thai medicinal plant Pueraria mirifica. A key step in
the first total synthesis of this compound by Corey and Wu
was the Lewis acid catalyzed cyclization of the tricyclic ketone
177 to form 178 (Scheme 37).[86] This reaction can be regarded as a
transannular double cation–olefin cyclization or as a Lewis acid
catalyzed, inverse-electron-demand intramolecular Diels–
Alder reaction. Interestingly, during the course of the total
synthesis of 176, approximately 1 mg of 179 was also isolated
from extracts of P. mirifica. It is thus possible that 179 is a
biosynthetic precursor to 176, in which case 176 may arise
through an inverse-electron-demand Diels–Alder cyclodo-
addition.

3.4.3. Pycnidione and Others

Pycnidione (180),[87] eupenifeldin (181),[88] and epolone B
(182)[89] are a group of recently isolated fungal metabolites
that possess identical tropolone rings attached to a sesqui-

Scheme 36. Proposed biosynthesis of ircinianin (172) and wistarin (175).[85]
terpene backbone. Biosynthetically, these compounds are proposed to arise from a hetero-Diels–Alder reaction of the C11-hydroxylated humulene \(183\) and quinone methide tropolone \(184\) (Scheme 38). The quinone methide \(184\) may in turn be generated by dehydration of the trihydroxy species \(185\). Cai et al. suggested epolone \(B\) \(182\) might be a biosynthetic precursor to pycnidione \(180\) through a second hetero-Diels–Alder reaction.\[89\]

To test this hypothesis, a model study was performed using humulene \(186\) and the benzotropolone \(187\) (Scheme 39). The benzotropolone \(187\) was formed from a thermal retro-Diels–Alder reaction of \(188\).\[90\] In situ trapping with humulene \(186\) afforded the Diels–Alder cycloadduct \(189\), which is analogous to epolone \(B\) \(182\). Addition of an excess of \(187\) at \(150^\circ\text{C}\) gave \(190\) as a 1:1 mixture of diastereomers. Since the naturally occurring Diels–Alder cycloadducts \(180–182\) are enantiomerically pure, there is a possibility that the addition of the tropolone is enzymatically catalyzed in the natural system.

A structurally similar compound, lucidene \(191\), has been isolated in racemic form from the root bark of \textit{Uraria lucida}.\[91\] It has been proposed as the product of a double [4+2] cycloaddition of \(o\)-benzoquinone methide \(192\) and \(\alpha\)-humulene \(186\), which is also a co-metabolite. A biomimetic synthesis provided the natural product \(191\) as well as the monoadduct \(194\) and isolucidene \(195\).\[92\] Unlike compounds \(180–182\), lucidene \(191\) is not optically active, thus it most likely arises from a non-enzymatic Diels–Alder reaction (Scheme 40).

4. Phenylpropanoids

4.1. Intramolecular Cycloadducts

4.1.1. Phenylphenalenones

Phenylphenalenones are characteristic pigments found in the monocotyledon family \textit{Tinctoria}. An early study on the biosynthesis of these compounds indicated that [2,\(^{14}\text{C}\)]tyrosine was incorporated specifically at C5 of the haemocorin aglycone \(196\).\[93\] A biosynthetic pathway was proposed (Scheme 41) that involves condensation of one molecule each of phenylalanine and tyrosine (or the metabolic equivalent) with one molecule of acetic acid and loss of a carboxy group to provide a diarylheptanoid intermediate \(197\). This intermediate could then cyclize, possibly through a Diels–Alder cycloaddition, to provide the phenylphenalene ring system. Further evidence for this pathway was the specific incorporation of [1,\(^{13}\text{C}\)]phenylalanine at C7 of the lachnanthiside aglycone \(198\).\[94\] Although phenylalanine and tyrosine were found to be precursors to the phenylphenalenones, other shikimate-derived phenylpropanoids, such as cinnamic
and coumaric acid, have also been determined to be precursors.\[95,96\]

It was not until 1995 that experimental evidence for the intermediacy of a diarylheptanoid in phenylphenalenone biosynthesis was obtained. Höschler and Schneider showed that the Diels–Alder precursor 199 was specifically incorporated into anigorufone (200) from feeding experiments with the cultured roots of *Anigozanthos preissii* (Scheme 42).\[96\] An earlier synthetic study showed that after oxidation with NaIO₄, unlabeled 199 was converted into lachnanthocarpone (202) spontaneously at room temperature through an intramolecular Diels–Alder cycloaddition.\[97\] Thus, the Diels–Alder cyclization leading to the phenylphenalenone ring system appears to be non-enzymatic.

### 4.1.2. Brombyins

The brombyins are novel decalin derivatives from the Australian tree *Brombya platynema* which are produced in nature in racemic form.\[98\] Although the metabolites 203 and 204 could be biogenetically derived from the oxidative coupling of two cinnamic acid residues (the 9-2', 7-7'-positions in 205, Scheme 43), this seems unlikely because of the perhydrogenated nature of one of the six-membered rings and the lack of optical activity of the natural products.

Instead, the isolation of the intermediate 206 led to the hypothesis of the linkage of a single C₆C₃ moiety 207 with an acetate chain to give an intermediate such as 208 (Scheme 43). A spontaneous intramolecular Diels–Alder cyclization of intermediate 209 could lead to two racemic products corresponding to 203 and 204.

### 4.2. Intermolecular Dimeric Cycloadducts

#### 4.2.1. Dimeric Coumarins

Another group of phenylpropanoid Diels–Alder adducts is represented by the dimeric coumarins (Scheme 44). The first dicoumarin discovered, thamnosin (212), was postulated to arise from the Diels–Alder cycloaddition of two molecules of the monomer 213.\[99\] Later, the dicoumarin toddasin (214, mexolide) was isolated from two different sources, *Toddalia asiatica* and *Murraya exotica*.\[100,101\] ESI mass spectrometry of 214 led to the formation of the retro-Diels–Alder fragment 215.\[100\] Treatment of mexoticin (216), a co-metabolite of 214 in *Murraya exotica*, with P₂O₅ in refluxing xylene led to the formation of 214, presumably through the dehydration product 215.\[101\] Toddacoumalone (217) was the first example of a mixed coumarin dimer. The CI mass spectrum of 217 showed the presence of protonated ions corresponding to the coumarin 218 and the quinolone 219.\[102\]

#### 4.2.2. Kuwanon J and Chalcomoracin

Other phenylpropanoids that are reportedly derived from a biological Diels–Alder cyclization are the metabolites kuwanon J (220) and chalcomoracin (221) from *Morus alba* L. Selection of callus cultures from *Morus alba* that

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*Scheme 41. Proposed biosynthesis of the phenylphenalenones.*\[97\]

*Scheme 42. Synthesis and biosynthesis of phenylphenalenones 200 and 202.*\[96,97\]

*Scheme 43. Proposed biosynthesis of the brombyins.*\[96\]
efficiently produce Diels–Alder-type adducts (ca. 100 times more of 220 and 221 than the intact plant) allowed biosynthetic studies to be performed on these compounds.[105] Feeding experiments with [1-13C]-, [2-13C]-, and [1,2-13C]-acetate revealed that 220 and 221 are formed from the condensation of two cinnamoylpolyketide-derived skeletons 222 (see Scheme 45).

The arylbenzofuran skeleton of 220 is apparently formed from a novel type of cyclization of the cinnamoylpolyketide 222 followed by decarboxylation to give 223.[103] Interestingly, [1-13C]-, [2-13C]-, and [1,2-13C]-acetate were not incorporated into the prenyl groups of 220 and 221, thus indicating that the isoprene groups are not derived through the usual mevalonate pathway.[103,104] Incorporation of [3-13C]-L-phenylalanine and [3,3-13C]-L-tyrosine into both halves of 220 and 221 suggests a common biosynthetic route to the cinnamoylpolyketide skeleton via p-coumarate.[105]

Addition of the O-methylated chalcone 229 to Morus alba cell cultures resulted in the formation of 230 as well as the O-methyl derivatives of kuwanon J (231) and chalcomoracin (232).[106] The structure of 230 indicates that prenylation occurs after aromatization of the cinnamoyl polyketide. Subsequent addition of 230 to M. alba cell cultures resulted in the formation of 231 and 232. This result strongly suggests that, in the natural system, one molecule of the prenylated chalcone is recognized as the dienophile (225) while another prenylated chalcone, after dehydrogenation, acts as the diene (226 or 228). Compounds 231 and 232 are optically active and possess the same configuration as 220 and 221, which suggests that the condensation reaction between these partners is enzymatic.[106] Close examination of the Diels–Alder-type adducts after [2-13C]-acetate feeding experiments with M. alba revealed that the adducts kuwanon V (233) and mulberrofuran (234) had a higher enrichment factor (24 and 22%, respectively) than either 220 or 221 (4 and 17%, respectively).[107] This result suggests that 220 and 221 are formed by hydroxylation of 233 and 234.

Biotransformation experiments with M. alba cell cultures were also used to determine the structure of the Diels–Alder adduct artontin (235, Scheme 46).[108] Since 235 is only a minor metabolite from the root bark of Artocarpus heterophyllus (0.7 mg), structure determination was difficult. The cooccurrence of artocarpesin (236) in the same plant led to the proposed biogenesis and structure shown in Scheme 46. To confirm this proposal, 236 was added to a M. alba cell culture. Work-up provided an aberrant metabolite (8 mg, 3.5 × 10^-6%) that had an identical mass and 1H NMR spectrum as 235. These results indicate that in M. alba cultures, 236 reacted as a diene and 237, which is produced in the cells, acted as a dienophile in the formation of the putative cycloadduct.

4.2.3. Asatone

Asatone (238) is a neolignan isolated from the stems and rhizomes of Asarum teitoneum Hayata.[109] The base peak in the mass spectrum of 238 was observed at half the molecular weight, which is consistent with a retro-Diels–Alder fragmentation. The skeleton of 238 is comprised of two C6C3 units, which biosynthetically can be envisioned as enzymatically oxidized 4-allyl-2,6-dimethoxyphenol (239, Scheme 47). The optically inactive dieneone 240 can then dimerize to provide 238 through an intermolecular Diels–Alder reaction. In fact, anodic oxidation of 239 in methanol provided a mixture of 240 and 248. 240 was quantitatively converted into asatone (238) upon standing at room temperature.[110] The related lignans heterotropatrine (241) and isoheterotropatrine (242) were postulated to be the Diels–Alder adducts of 238 and 240.[111]

5. Alkaloids

5.1. Daphniphyllum Alkaloids

The daphniphylline alkaloids are a growing class of polycyclic natural products that were first isolated in 1909 from the deciduous tree Yuzurha (Daphniphyllum macroptum). The four different skeletal classes of daphniphylline alkaloids are represented by daphniphylline (243), secodaphniphylline (244), yuzurimine (245), and daphnilactone A.
Early work on the biosynthesis of daphniphylline (243) established its mevalonate origin via a squalene-like intermediate.[112] Later, Ruggeri and Heathcock devised a biosynthetic proposal for the construction of the complex polycyclic ring systems of the daphniphyllum alkaloids through a hetero-Diels–Alder cyclization (see Scheme 48).[113,114]

They proposed that the squalene-derived dialdehyde 247 might condense with pyridoxamine to provide the azadiene 248. A prototropic shift in 248 would give 249, which upon nucleophilic addition of an amine (possibly from lysine) would furnish the enamine 250. An intramolecular enamine/enal cyclization of 250 would afford a bicyclic dihydropyran derivative 251. A process of proton-mediated addition and elimination would then provide the dihydropyridine derivative 255. A catalyzed intramolecular hetero-Diels–Alder reaction of 255 would give the tetrahydropyridine 256.
Subsequent enelike cyclization of \(256\) would give the penta-cyclic compound proto-daphniphylline \((257)\), a proposed precursor to daphniphylline.

To explore the proposed biosynthesis, Heathcock et al. completed a biomimetic total synthesis of \((257)\) (see Scheme 49).\([115]\) The synthesis utilizes a one-pot procedure that was also used to synthesize five daphniphyllum alkaloids.\([115,116]\) Oxidation of the 1,5-diol \((258)\) to the dialdehyde \((259)\) was accomplished through a Swern oxidation. The crude reaction mixture was treated with ammonia followed by acetic acid and ammonium acetate to provide the azadiene \((261)\). An intramolecular Diels–Alder reaction furnished the imine \((256)\). Heating the acetic acid solution of the imine then facilitated an intramolecular aza–Prins cyclization and gave \((257)\).

5.2. Indole Alkaloids
5.2.1. Iboga/Aspidosperma Alkaloids

The iboga and aspidosperma alkaloids are perhaps the most well-known examples of natural products potentially arising from a biosynthetic Diels–Alder reaction, and yet there is still no definitive proof for this biosynthetic pathway. By 1970, Scott had elucidated a significant portion of the biosynthetic pathway through hydroponic feeding experiments with \(Vinca rosea\) shoots.\([117]\) These results along with chronological isolation studies led to the proposed biosynthetic pathway outlined in Scheme 50. The intermediacy of \((271)\) was invoked to explain the incorporation of stemmadeanine \((272)\) into both catharanthine \((273)\), iboga skeleton) and into vindoline \((274)\), aspidosperma skeleton). Scott proposed that heterolytic ring opening and concomitant dehydration of stemmadeanine \((272)\) would lead to the formation of dehy-drosecodine \((271)\), which could undergo two possible \([4+2]\) cycloaditions. If the 2-dihydropyridine system of \((271)\) behaved as a diene, then \((273)\) would be formed. However, with the exception of biomimetic syntheses,\([118]\) no direct biosynthetic evidence for this provocative postulate has been obtained.

5.2.2. Manzamine Alkaloids

The manzamines are a growing group of cytotoxic marine sponge alkaloids that possess unusual polycyclic diamine skeletons. Among this group are manzamine A \((275)\) and B \((276)\).\([119]\) Ircinal A \((277)\) and B \((278)\),\([120]\) ircinol A \((279)\) and B \((280)\),\([121]\) keramaphidin B \((281)\) and xestocyclamine \((282)\),\([123]\) and ingenamine.\([124]\) The ircinals \((277)\) and \((278)\) were proposed to be biosynthetic precursors to the manzamines \((275)\) and \((276)\).\([120]\) In fact, \((277)\) was chemically transformed to \((275)\) through a Pictet–Spengler cyclization with tryptamine and subsequent oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).\([120]\) Ircinols \((279)\) and \((280)\) are antipodes of the alcoholic forms of \((277)\) and \((278)\) and represent the first alkaloids in this class of compounds to possess the opposite absolute configuration to that of the manzamines.\([119]\) Keramaphidin B \((281)\) was also postulated as a manzamine biosynthetic precursor through formation of an ircinal from hydrolysis the N2–C3 bond of the imino form of \((281)\).\([122]\)

In 1992, Baldwin and Whitehead outlined an elegant unified biogenesis for the manzamines (Scheme 51).\([125]\) Manzamine B \((276)\) was envisioned to be derived from four building blocks: ammonia, the C10 unit \((283)\), a C3 unit (acrolein), and tryptophan. The key step for the proposed biogenesis was an intramolecular \(endo\)-Diels–Alder cycloaddition of a partially reduced bispyridinium species \((284) \rightarrow \text{Other intermediate} \rightarrow \text{Final product})\). Later, Baldwin et al. expanded their biosynthetic proposal to include \((281)\).\([127]\) Baldwin, et al., completed a biomimetic total synthesis of keramaphidin B \((281)\) by dissolving the proposed intermediate \((284)\) in a methanol/Tris buffer solution followed by the other hand, the 2-dihydropyridine ring of \((271)\) reacted as a diene, then \((273)\) would be formed. However, with the exception of biomimetic syntheses,\([118]\) no direct biosynthetic evidence for this provocative postulate has been obtained.
reduction with NaBH₄ to provide a small amount of 281 (Tris = tris(hydroxymethyl)aminomethane). The low yield of 281 was rationalized as a reflection of the inclination of intermediate 285 to disproportionate. The researchers further argued that in vivo, a Diels–Alderase could limit the conformational mobility of the substrate, which would not only minimize the change in entropy toward the transition state but would also obviate the intrinsic problem of disproportionation.

Marazano and co-workers proposed an alternate route for the biosynthesis of the manzamines in 1998. They suggested that the biosynthetic Diels–Alder reaction could involve a substituted 5-amino-2,4-pentadienal as the diene thereby avoiding the problem of disproportionation and implementing less ring strain during the [4+2] cycloaddition. In this proposal, the building blocks of malondialdehyde (instead of acrolein), ammonia, and an appropriate unsaturated dialdehyde would generate the macrocycle 290. From 290, two possible routes were proposed (Scheme 52). Analogous to the model of Baldwin et al., reductive cyclization of 290 would lead to the dihydropyridinium species 291. Diels–Alder cycloaddition followed by reduction of 292 would directly produce the proposed manzamine precursor, aldehyde 288. Alternatively, direct cyclization of 290 to give intermediate 293, followed by reduction of the resulting imine functionality and cyclization would provide the enamine 294, which presumably could lead to manzamine B (276). The occurrence of the proposed enamine 294 was justified by the isolation of the manzamine dimer, kauluamine, isolated from an Indonesian sponge[130].

To test the first of these alternate biosynthetic routes, Marazano and co-workers performed a model synthesis as shown in Scheme 53. However, the cycloaddition of the salt 295 with 296 only produced the amino ester 297. Presumably intramolecular hydridetransfer occurred to give 298 followed by hydrolysis of the resultant imine 299.
Scheme 50. Proposed biosynthesis of the iboga and aspidosperma alkaloids.\textsuperscript{[117]}

Scheme 51. Proposed biosynthesis by Baldwin et al. of manzamine B (276).\textsuperscript{[127]}

Scheme 52. Proposed biosynthesis by Marazano and co-workers of manzamine B (276).\textsuperscript{[129a]}

Brevianamides

Birch, Wright, and Russel first isolated the fungal metabolite brevianamide A (301) from *Penicillium brevicompactum* as well as other minor metabolites including brevianamide B (302).\[131\] In 1970, Sammes proposed that the unique bicyclo[2.2.2]diazaoctan ring system of brevianamide A originated from a hetero-Diels–Alder cycloaddition reaction from 300.\[132\] This hypothesis was evaluated experimentally by treating the model dihydroxypyrazine 303 with dimethyl acetylenedicarboxylate (304) and with norbornadiene (305) to provide the Diels–Alder cycloadducts 306 and 307, respectively (Scheme 54).

Early radiolabeling and feeding experiments performed by Birch and co-workers indicated that tryptophan, proline, and mevalonic acid were precursors to brevianamide A (Scheme 55).\[133\] This same study also revealed the incorporation of isotopically labeled brevianamide F (308) into 301 in significant radiochemical yield. From these results, it was postulated that the reverse-prenylated intermediate deoxybrevianamide E (309) was a biosynthetic precursor. Deoxy-

\[\text{Scheme 53. Model study for the proposed biosynthetic Diels–Alder cycloaddition of the manzamine alkaloids.}\[129b\] CSA = camphorsulfonic acid.\]

\[\text{Scheme 54. Proposed and a model study by Porter and Sammes of the biosynthesis of brevianamide A (301).}\[132\]\n
Brevianamide E (309) was isolated from the austamide (310) producing fungus, *Aspergillus ustus*, but it has not been detected as a free metabolite from brevianamide-producing cultures. In 1993 Williams and co-workers performed feeding experiments with [8-3H2]-309 that provided strong experimental evidence that 309 was a biosynthetic precursor to 301 and 302.\[134\]

Williams and co-workers completed the first asymmetric total synthesis of (−)-brevianamide B (302), which revealed an unusual enantiomorphous relationship between 301 and 302 with respect to the bicyclo[2.2.2]diazaoctan nucleus.\[135\] Based on these results, the biogenesis outlined in Scheme 56 was postulated. According to this proposal, two-electron oxidation of 309 would yield the azadiene 311, which would suffer intramolecular hetero-Diels–Alder cycloaddition to form the enantiomeric hexacyclic cycloadducts 312 and 313. Finally, R-selective oxidation of the indole at the 3-position and a pinacol-type rearrangement would provide 301 and 302. Feeding experiments were performed with *Penicillium brevicompactum* using the proposed synthetic 13C-labeled intermediates 312 and 313, yet no detectable incorporation was observed.\[136\] In addition, efforts to identify compounds 312 and 313 as natural metabolites of *Penicillium brevicompactum* failed to produce any evidence for these substances. Although these results do not rigorously exclude the biosynthetic
intermediacy of 312 or 313, it led to the proposal of an alternate biosynthetic pathway as illustrated in Scheme 57.\[134\]

In the new proposal, 309 was envisaged to undergo oxidation to the hydroxyindolenine 316 and pinacol rearrangement to the indoxyl 317 before forming the requisite azadiene 319 through two-electron oxidation and enolization. The intermediacy of 316 was supported by the isolation of the co-metabolite brevianamide E (320), which was shown to be a shunt metabolite formed by irreversible nucleophilic ring closure via 316. It was demonstrated that [8-\textsuperscript{3}H\textsubscript{2}]-309 was incorporated into 320 in high radiochemical yield (38.5% specific incorporation).\[134\] However, tritium-labeled 320 when re-fed to cultures of \textit{P. brevicompactum} resulted in no significant radiochemical labeling of either brevianamides A or B, thus indicating that brevianamide E is indeed a shunt metabolite. Based on these findings, it was speculated that the hydroxyindolenine 316 could suffer two fates: 1) irreversible ring closure to brevianamide E or 2) pinacol-type rearrangement to 317 leading ultimately to brevianamides A and B. The natural products 301 and 302 would thus arise from the putative intramolecular hetero-Diels–Alder cyclodehydration of the azadiene 319. For this hypothesis to be valid, 319 must form a major conformer 319a that results in the formation of 301 and a minor conformer 319b produces 302. It is also possible that 316 is oxidized to the corresponding azadiene prior to the pinacol-type rearrangement, wherein the intramolecular Diels–Alder reaction would give the two diastereomeric hydroxyindolenine precursors to 301 and 302. This possibility has not yet been experimentally tested.

\[\text{Ab initio calculations were carried out to determine if there was a conformational predilection of the azadiene 319.}^{[136]}\] The potential energy barriers for the four possible diastereomeric transition-state structures A, B, A', and B' were calculated (6-31G*/3-21G; Scheme 58). The potential energy barrier for A was determined to be 38.68 kcal mol\textsuperscript{-1} and the potential energy barriers for B, A', and B' were higher by 6.35, 11.02, and 12.73 kcal mol\textsuperscript{-1}, respectively. While the transition-state structures A and B lead to the observed biosynthetic products 301 and 302, respectively, the transition-state structures A' and B' lead to diastereomers 321 and 322, respectively, which are unknown as natural products. The positioning of the vinyl group in relation to the azadiene system may cause the difference in energy between the four transition states, and the difference between A and B was rationalized by the capacity of A to access an intramolecular hydrogen bond between the indoxylamino group and the oxygen atom of the amide carbonyl group. This ab initio study is consistent with the observed product ratios of 301 and 302.
and supports the proposal of an intramolecular Diels–Alder cycloadDITION of the proposed key biosynthetic intermediate 319. However, the issue of enzymatic catalysis or protein organization of the pretransition state conformers remains unsolved.

Although the biogenesis of the brevianamides was first postulated to occur through a biosynthetic Diels–Alder cycloadDITION in 1970 by Porter and Sammes,[132] there was very little published data on the reactivity of such azadienic systems until recently.[137] To explore the feasibility of an intramolecular [4+2] reaction for the construction of the bicyclo[2.2.2]diazaoctane, a biomimetic total synthesis of brevianamide B (302) was completed by Williams and co-workers (Scheme 59).[138] Treatment of epi-deoxybrevianamide E (323) with trimethylxonium tetrafluoroborate provided the lactim ether 324. Subsequent oxidation with DDQ gave the azadiene 325, which cyclized spontaneously upon tautomerization under aqueous basic conditions to furnish the racemic, diastereomeric cycloaducts 327 and 328 in a 2:1 ratio (90% combined yield) favoring the unnatural syn product 327.

Separate diastereoselective oxidations of 327 and 328 with m-CPBA provided the hydroxyindolenines 329 and 330, respectively. Finally, base-catalyzed pinacol-type rearrangements and removal of the lactim ethers provided racemic C19-epi-brevianamide A (331) and racemic brevianamide B (302). This study demonstrated that the core bicyclo[2.2.2]diazaoctane can indeed arise through an intramolecular Diels–Alder cyclization of the unactivated isoprene-derived dienophile and an azadiene system structurally and electronically similar to that proposed for the biosynthesis in water under ambient conditions. However, the stereoselectivity in the biosynthetic system which exclusively favors formation of the anti product, was not mirrored in the laboratory cyclization which favored the syn configuration at C19. These results raise the possibility of protein organization of the pretransition state conformations of the substrate, but leave uncertainty as to the oxidation state of the indole moiety (indole, hydroxyindolenine, or indoxyl).
5.2.4. Paraherquamides

The paraherquamides (332–344) are a group of heptacyclic mycotoxins isolated from various *Penicillium* sp. and *Aspergillus* sp. Structurally, the paraherquamides are similar to the brevianamides, and vary with respect to the substitution and oxygenation in the proline and the prenylated oxindole rings. The first member of this family of spirooxindoles to be discovered was paraherquamide A (332), isolated in 1980 from *Penicillium paraherquei*. Subsequently, paraherquamides A–G (332–335, 338, 341, 342) were isolated from *Penicillium charlesii* (332), *E* (338), *F* (341), and *G* (342) were also isolated from a *Penicillium* sp. (IMI332995) found in the soil of Kemer, Turkey. Several related compounds, including VM55595 (333), VM55596 (336), VM55597 (337), and VM55599 (344), were also isolated from this strain. VM55599 (344) is the only member of the family that contains an indole ring instead of an oxindole ring. The most recent additions to the paraherquamide family are SB203105 (339) and SB200437 (340), which were isolated in 1998 from an *Aspergillus* sp. (IMI337664).

Through feeding experiments, Williams and co-workers determined that L-methionine, L-tryptophan, and L-isoleucine were the proteinogenic amino acid building blocks, which give rise to paraherquamide A (332), Scheme 60. Incorporation of isotopically labeled L-isoleucine revealed that it was the source of the unusual nonproteinogenic amino acid β-methylproline (which is later converted into β-methyl-β-hydroxyproline) through a four-electron oxidation/two-electron reduction sequence with retention of the pro-S hydrogen atom at C16 (paraherquamide numbering). Additional feeding experiments with [13C2]acetate and [13C6]glucose revealed the mevalonate origin of the isoprene moieties (C19–C23 and C24–C28) of 332.

Interestingly, the feeding experiments indicated that *Penicillium fellutanum* constructs each isoprene-derived quaternary center in 332 by disparate stereochemical pathways. The quaternary center in the dioxepin ring (C24–C28) was found to be formed in a completely stereospecific manner. However, scrambling of the 13C label into both of the gem-dimethyl groups was observed in the quaternary center of the bicyclo[2.2.2]diazaoctane portion (C19–C23), thus indicating that the stereochemical integrity of the acetate-derived 13C label in the dimethylallyl pyrophosphate moiety was sacrificed in the construction of this quaternary center.

Williams and co-workers proposed that a “reverse” prenyltransferase catalyzes a nonface-selective intermolecular S_{2}′-type addition of the dimethylallyl pyrophosphate moiety to the 2-position of the tryptophan-derived indole ring, thus scrambling the Z^{13}C label in the isoprene moiety (Scheme 61). Analogous to the proposed biosynthesis for the brevianamides, it was anticipated that this reverse-prenylated moiety 351 would undergo a [4+2] cycloaddition reaction across the α carbon atoms of L-tryptophan and (S)-methyl-L-proline and eventually lead to paraherquamide A (332).

As in the case of the brevianamides, it was unclear whether oxidation of the tryptophan moiety occurred before or after the putative hetero-Diels–Alder reaction. Isolation of the hexacyclic indolic metabolite VM55599 (344) from the paraherquamide-producing *Penicillium* sp. (IMI332995) suggested that the tryptophan oxidations occur after the construction of the bicyclo[2.2.2] ring system. However, the relative stereochemistry of C14 and C20 in 344, as determined by Everett and co-workers by extensive 1H NMR NOE data, was found to be opposite to that found in 332. If the β-methylproline ring of 344 was derived, as in the case of 322, from (S)-isoleucine, then cycloaddition would have to occur from the seemingly more hindered face of the azadiene system (B in Scheme 62) with the methyl group of the β-methylproline ring syn to the bridging isoprene moiety. If, on the other hand, cycloaddition occurs with the methyl group of the β-methylproline ring anti to the bridging isoprene unit (A in Scheme 62), then an intermediate would be formed which could lead to all of the paraherquamides containing a β-methylproline moiety. Indirect support for the hypothesis that
Feeding experiments with the $^{13}$C-labeled cycloadducts revealed no incorporation in ($\pm$)-344, its oxidized counterpart ($\pm$)-352, or the diketopiperazine ($\pm$)-353 (Scheme 64). However, significant incorporation of ($\pm$)-354, the C14 epimer of 344, into paraherquamide A (332) was observed by $^{13}$C NMR spectroscopy and from analysis of the electrospray mass spectrum (0.72% incorporation). These results indicate that the formation of the bicyclo[2.2.2]diazaoctane ring system occurs at the stage of the nonoxidized

*Penicillium fellutanum.*$^{[147]}$ Syntheses of these labeled substrates were accomplished through a biomimetic intramolecular Diels–Alder cycloaddition strategy as illustrated in Scheme 63.$^{[146,147]}$ Interestingly, the laboratory cycloaddition slightly favored (1.47:1) the approach of the dienophile from the same face as the methyl group in the $\beta$-methyl proline ring, which leads to the relative stereochemistry of 344. Additionally, the ratio of the anti to syn isomers at C20 was approximately 1:2.4 in favor of the natural configuration. As in the related cycloaddition reaction of 326 (Scheme 59) the poor facial bias of the laboratory Diels–Alder reaction strongly hints that protein organization of the pretransition state conformers might be operative in the biosynthesis of paraherquamide A.

The major cycloaddition pathway passes through conformer A was based on the small amount of 344 isolated from *Penicillium* sp. (IMI332995) cultures (344:332 ca. 1:600).$^{[146]}$

To determine if 344 was an intermediate in the biosynthesis of 332, as initially postulated by Everett and co-workers, Williams and co-workers prepared $[13$C$_2]$($/C_6$)-344, the oxidized form of $[13$C$_2]$($/C_6$)-352 as well as the alleged $^{13}$C-labeled paraherquamide progenitors 353 and 354, and examined these substances as potential pathway metabolites in

Scheme 6a. Incorporation of amino acids into paraherquamide A (332).$^{[143]}$
tryptophanyl moiety (namely, indolyl). These results provide additional evidence that 344 is a minor shunt metabolite of the paraherquamide pathway. Moreover, these results document the intermediacy of an advanced metabolite 354, potentially formed by an intramolecular hetero-Diels–Alder cycloaddition, which contains the core structural elements of the paraherquamide framework prior to a series of oxygenation reactions.

Very recently, Sanz-Cervera and Williams completed an asymmetric biomimetic total synthesis of (−)-344 that served to unambiguously establish the absolute stereochemistry of this substance (Scheme 65). As predicted by these authors in the unified biogenesis proposal depicted in Scheme 62, 344 retains the (S)-Ile stereochemistry in the β-methylproline ring and consequently, has a bicyclo[2.2.2]-diazaoctane ring system that is enantio- morphic to that embedded in the paraherquamides.

It was quite surprising to observe that cycloadduct 353, which contains the relative and absolute stereochemistry of the

Scheme 62. Proposed unified biogenesis of paraherquamide A (332) and VM55599 (344).
The cycloadducts obtained in the previously reported racemic synthesis gave (as lactim ethers) compounds stereochemically corresponding to \(352:374:375:353\) in a ratio of 3.7:1.6:1:2.6. In the present case, the ratio is 3.5:1.5:1.0. In the biological system, the diastereomeric distribution is expressed as > 600:1 (corresponding to \(332:344\)) as evidenced by the complete lack of natural metabolites that would arise from substances containing the stereochemistry imbedded in either \(374\) or \(375\). The laboratory intramolecular Diels–Alder cycloadditions described in Schemes 63 and 65 again demonstrate an unexpected proclivity for the formation of the relative stereochemistry of \(344\). This result is in sharp contradiction to the stereochemical preference expressed in nature. Although the oxidation state of the putative azadiene species (A/B, X = O or H2 in Scheme 62) in the biological system currently remains uncertain. The contrast between the two biomimetic laboratory cycloaddition reactions and that paraherquamides, was not detected from the cycloaddition reaction. The cycloadducts obtained in the previously reported racemic synthesis gave (as lactim ethers) compounds stereochemically corresponding to \(352:374:375:353\) in a ratio of 3.7:1.6:1:2.6. In the present case, the ratio is 3.5:1.5:1.0. In the biological system, the diastereomeric distribution is expressed as > 600:1 (corresponding to \(332:344\)) as evidenced by the complete lack of natural metabolites that would arise from substances containing the stereochemistry imbedded in
6. Addendum

Since submission of this manuscript, several recent papers appeared on the biomimetic total synthesis of FR182877 (376) and longithorone (163). As these works have significant implications concerning the biosynthesis of these natural products, the key findings will be briefly described.

FR182877

Two impressive biomimetic total syntheses of FR182877 (376) have appeared[149,150] that provide experimental support for a provocative biogenetic proposal originally suggested by Sorensen and co-workers in 1999.[151] The construction of FR182877 (formerly known as WS9885B) could arise by a cascade of cyclization reactions (Scheme 66) involving: a) an intramolecular Diels–Alder cycloaddition from the polyketide 377 to 378, b) an intramolecular Knoevenagel cyclization (to 379), and c) a transannular hetero-Diels–Alder cycloaddition to directly furnish 376.

In 2001, Sorensen and co-workers suggested a slight revision of this elegant biogenesis wherein the related polyketide substrate 380 would undergo an intramolecular Knoevenagel cyclization (to 381). Successive transannular Diels–Alder and transannular hetero-Diels–Alder cycloadditions then directly furnished 376 (Scheme 67).[151b]

This strategy inspired a biomimetic total synthesis of this natural product[149] that lends strong, albeit indirect, experimental support for the biogenetic hypothesis. The key features of the total synthesis of (+)-FR182877 by Sorensen and co-workers are illustrated in Scheme 68.

Evans and Starr also reported a biomimetic cyclization cascade to (−)-376 (Scheme 69).[150] This study also confirmed the absolute configuration of this natural product.[152] The biosynthesis of the related natural product hexacyclic acid (390)[153] may arise from an alternative exo conformer of a related polyketide-derived macrocycle.

Longithorone

Shair and co-workers recently completed an elegant and impressive biomimetic total synthesis of longithorone (163). These researchers exploited an interesting chirality-transfer strategy that involved the use of stereogenic centers to control the atropisomerism followed by removal of the stereogenic centers, and, transfer of the atropisomerism chirality back to the stereogenic centers.
Schmitz and co-workers proposed a provocative biogenesis of longithorone wherein an intermolecular Diels–Alder reaction between [12]paracyclophanes \(391\) and \(392\) form ring E and a subsequent transannular intramolecular Diels–Alder reaction across \(391\) forms rings A, C, and D (Scheme 70).\(^{[78a]}\) Shair and co-workers have capitalized on this hypothesis in a beautiful total synthesis of \((-\)\)-376 (Scheme 71).\(^{[78a]}\)

**Scheme 68.** Biomimetic total synthesis of \((+)-376\) by Sorensen and co-workers.\(^{[149]}\) DMAP = dimethylaminopyridine, EDC = 1,2-dichloroethane, PPTS = pyridinium \(p\)-toluenesulfonate, TES = triethylsilyl.

**Scheme 69.** Biomimetic total synthesis of \((-\)\)-376 by Evans and Starr.\(^{[150]}\) TBS = tert-butyldimethylsilyl.

**Scheme 70.** Biogenesis of longithorone A (163) as proposed by Schmitz and co-workers.\(^{[78a]}\)

**Scheme 71.** Biogenesis of longithorone A (163) as proposed by Evans and Starr.\(^{[150]}\) TBS = tert-butyldimethylsilyl.

7. **Summary**

The rapidly accumulating body of literature in this field that has been summarized in this Review suggests that nature indeed utilizes the Diels–Alder construction to generate a complex array of natural products. In many cases, such as in the endiandric acids,\(^{[49]}\) lucidene (191),\(^{[92]}\) and asatone (238),\(^{[110]}\) current experimental evidence argues that the putative biosynthetic Diels–Alder cyclization reactions are not enzyme-mediated, but occur spontaneously in the producing organism in a stereorandom fashion and give rise to racemic products. For the natural products that are enantiomerically pure, there is growing evidence that the Diels–Alder reactions might be enzyme-mediated. The experimental evidence for enzyme involvement is, however, circumstantial for virtually all of these systems. For example, the biomimetic laboratory cyclizations of the epolone B (182)\(^{[90]}\) and VM55599 (344)\(^{[146]}\) systems were not stereoselective.
which indicates that there may be some protein organization of the pretransition state conformations in the natural system.[114] The three systems in which the strongest and most direct experimental support for the existence of a Diels–Alderase resides are: a) the enzymatic activity observed for cell-free extracts of *Alternaria solani*.[21] that leads to the production of solanapyrone A (11), b) lovastatin nonaketide synthase[7] that leads to the production of lovastatin, and c) macrophomate synthase[8] that leads to the production of macrophomomic acid (113). Despite the impressive and difficult experimental work that the authors of these publications have gathered, in the quest for proving the existence of a Diels–Alderase, rigorous proof that the purified or partially purified (in the case of solanapyrone) proteins are catalyzing (that is, accelerating the rates of these reactions relative to the uncatalyzed systems) the pericyclic Diels–Alder reaction remains to be rigorously established. A great deal of difficult biophysical and kinetic work needs to be done on these systems to ascertain if true catalysis occurs, as measured by the classical tests of rate acceleration and stereoselectivity versus the substrate conversions to product in the absence of enzyme.

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