Diels – Alderases

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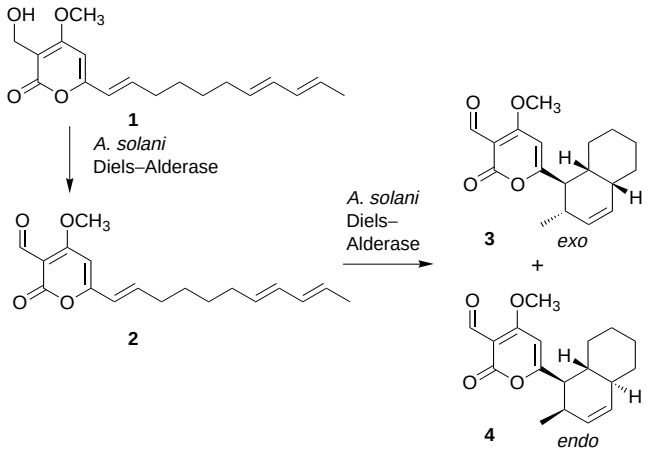
Only seven years ago Laschat asked, in a Highlight in *Angewandte Chemie*: "Does nature know the Diels – Alder reaction?".[1] In the meantime sufficient progress has been made for us to answer this question with a clear yes. The new discoveries in the study of natural Diels – Alderases, together with the impressive progress in our mechanistic understanding of catalytic antibodies which accelerate this class of reactions, are recounted here.

Diels – Alder reactions are concerted [4+2] cycloadditions of 1,3-dienes with electron-deficient alkenes to form carbocycles, according to the rules of pericyclic reactions. Despite the fact that this reaction is among the most important tools in synthetic organic chemistry, its involvement in the biosynthesis of natural products had never been secured. Based on structural properties of numerous natural products, the involvement of a Diels – Alder reaction in their biosynthesis was proposed[2] but, until recently, only indirect indications were given for the existence of Diels – Alderases.

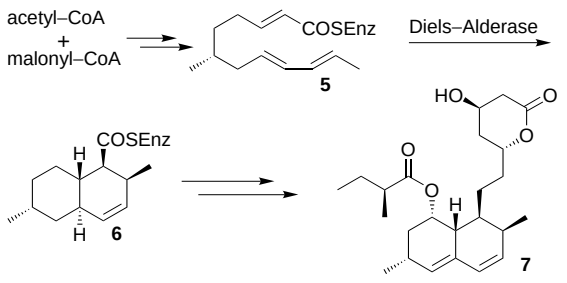
Experiments with cell-free extracts of the fungus *Alternaria solani* gave the first evidence for the involvement of naturally occurring Diels – Alder reactions.[3] Compared to control experiments, cyclisation of achiral linear **2** to form optically active (−)-solanapyrones A (**3**; *exo*) and D (**4**; *endo*) occurred with higher *exo* selectivity in the presence of the extracts (Scheme 1). Since this initial discovery, a team of chemists and biochemists at Hokkaido University has provided further evidence for the enzyme catalysis of this cyclisation by partially purifying the Diels – Alderase.[4] Incorporation of deuterium-labelled precursors showed that the intact diene – dienophile chain of **2** was transformed into the natural [4+2] adducts.[5]

First proof for an enzyme-catalysed Diels – Alder reaction with a pure enzyme was reported for the biosynthesis of the fungal polyketide lovastatin **7**. The decalin ring system of **7** is derived from a [4+2] cycloaddition of an intermediate hexaketide **5** as depicted in Scheme 2. After heterologous expression and purification to homogenity, the 335-kDa protein, lovastatin nonaketide synthase (LNKS), was obtained for functional analysis.[6, 7] LNKS was able to catalyse the Diels – Alder cyclisation of the synthetic thioester **8** to give a product spectrum different from that obtained during the uncatalysed cyclisation (Scheme 3). While thermal rearrangement of **8** gave a 1:1 ratio of the *exo* and *endo* adducts **9** and **10**, purified LNKS caused cyclisation to form the *endo* product **12** (ratio of **9**:**10**:**12**, 15:15:1), with stereochemistry corresponding to lovastatin **7**.[6] Formation of **12** requires a transition state having the methyl group in a crowded pseudoaxial arrangement, and it is speculated that LNKS supports this mechanism through van der Waals interactions.

Detailed mechanistic investigation of the complex five-step enzymatic transformation to form macrophomic acid **15** again suggested the involvement of a



Scheme 1. *Diels – Alder reaction in the biosynthesis of the solanapyrones A (**3**) and D (**4**).*



Scheme 2. *Proposed biosynthetic pathway for the formation of lovastatin **7**.*

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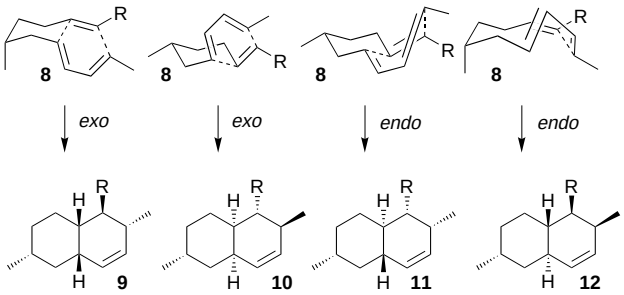
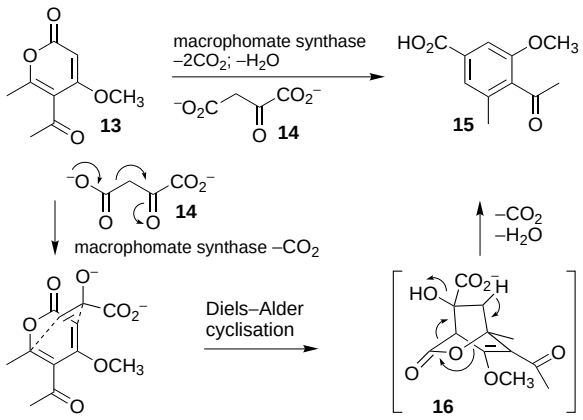


Scheme 3. *Spontaneous and enzyme-catalysed cyclisation of the* N*-acetylcysteamine thioester* ***8****. Only* ***9*** *and* ***10*** *are detected in the uncatalysed reaction. Product* ***12*** *is observed only in the presence of LNKS. R =* $COS(CH_2)_2NHCOCH_3$.

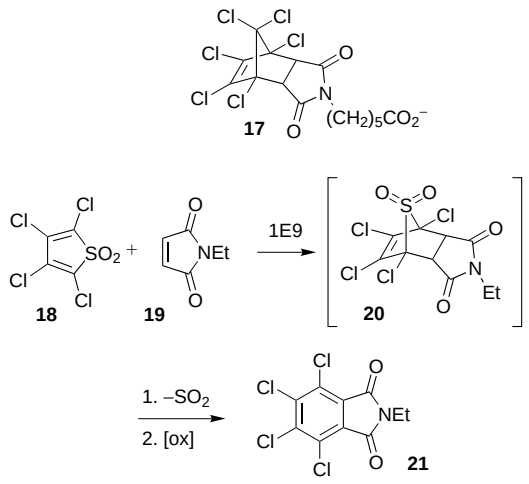
Diels – Alder reaction.[8] The formation of **15** from pyrone **13** and oxalacetate **14** is catalysed by macrophomate synthase involving two decarboxylations, two C–C bond formations and one dehydration (Scheme 4). The individual steps of this reaction sequence could be elucidated by careful stereochemical and kinetic characterisation and by use of synthetic substrate analogues. The suggested route is more likely than other possible transformations involving, for example, a Michael – aldol route from **13** to **15**.[8] Interestingly, all the natural enzymes described above catalyse a preceding reaction before the actual [4+2] cycloaddition. Thus, solanapyrone synthase catalyses the oxidation of prosolanapyrone II, LNKS the nonaketide synthesis and macrophomate synthetase the decarboxylated enolisation, before these enzymes catalyse the Diels – Alder reaction.

While our mechanistic understanding of naturally occurring Diels – Alder reactions is still in its infancy, detailed information on protein-catalysed [4+2] additions has been obtained with catalytic antibodies. If the immune system is challenged with suitable small molecules (haptens), it produces antibodies which recognise these structures. When haptens resemble reaction transition states, the corresponding antibodies can show catalytic activity for the respective transformation.[9] Antibodies against bicyclic compounds mimicking the highly ordered transition state of Diels – Alder reactions can catalyse cycloadditions by supporting the orientation of the substrates in a defined pocket.

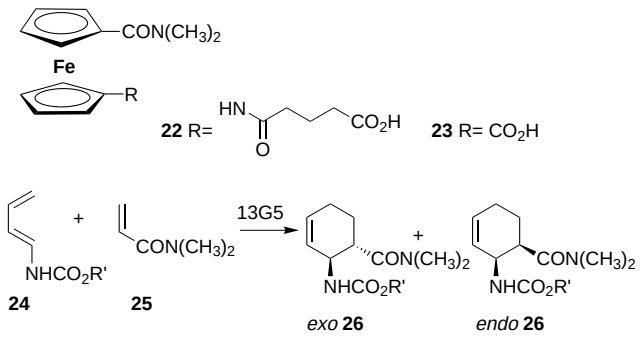
The most efficient Diels – Alderase antibody known to date has been generated against the hexachloronorbornene hapten **17**.[10, 11] It catalyses the bimolecular [4+2] cycloaddition of **18** and **19** with a $k_{cat.}/k_{uncat.}$ value in excess of 100 M (Scheme 5). The initially formed adduct **20** eliminates sulphur dioxide to give the planar compound **21** after air oxidation. This spontaneous geometric change prevents product inhibition and warrants multiple turnovers. Structural and theoretical studies of antibody 1E9 revealed almost perfect shape complementarity of the hydrophobic binding site for the transition state. The hapten is 86.3% buried in a hydrophobic pocket containing only two polar residues: Asn H35, which assists in the hapten orientation, and Ser L91, which is directed away from the ligand. Antibody 1E9 thus appears ideally suited for the preorganisation of the substrates in a reactive orientation to give **20**. But, despite the fact that the binding pocket of 1E9 is organised to maximise shape complementarity through 121 van der Waals contacts and π-stacking interactions, it does not appear to function as a classic entropy trap. Moreover catalysis is achieved entirely by reducing the enthalpy of activa-



Scheme 4. *Proposed five-step transformation of pyrone* ***13*** *catalysed by macrophomate synthase.*



Scheme 5. *The hexachloronorbornene derivative* ***17*** *served as a hapten to elicit antibody 1E9, which catalyses the Diels – Alder condensation of* ***18*** *and* ***19****. The intermediate* ***20*** *eliminates* SO_2 *and is air oxidised to form the planar compound* ***21****.*



Scheme 6. *The configurationally flexible ferrocene* ***22*** *served as a hapten for the generation of antibody 13G5, which catalyses the disfavoured* exo *Diels – Alder reaction to form* ***26****. R′ = 4-carboxybenzyl.*

tion from 15.5 kcal mol^{-1} (uncat.) to 11.3 kcal mol^{-1} (cat.) with the entropy of activation remaining around -22 cal K^{-1} mol^{-1} in both cases.[11]

An unusual hapten design was employed for generation of antibody 13G5, which catalyses the disfavoured *exo* Diels – Alder transformation to form **26**.[12] Heine et al. did not use a rigid bicyclic transition-state analogue but relied on the ferrocene derivative **22** as the hapten (Scheme 6). It is remarkable that this conformationally highly flexible hapten, with the cyclopentadiene rings rotating freely in solution, can be used to generate an efficient catalytic antibody. Apparently, the immune system can select a conformer that mimics the Diels – Alder transition state resulting in *exo* product formation. Antibody 13G5 catalysed the reaction to the *ortho* product **26** with high regio-, diastereo-, and enantioselectivity (> 98 % *exo*, 95 % *ee*), while the uncatalysed reaction is only regioselective, with a ratio of *ortho-endo* to *ortho-exo* products of 85:15. The crystal structure of 13G5 in complex with the ferrocenyl inhibitor **23** showed that the hapten is deeply buried (99 %) in the antibody binding site, forming 3 hydrogen bonds and 45 van der Waals interactions. Ab initio calculations suggest that two hydrogen-bonding interactions with diene **24** and the Lewis acid effect of a tryptophan on the dienophile **25** are activating and orienting the substrates.

Another strategy to achieve catalysis of Diels – Alder transformations by biomacromolecules involves catalytically active RNA. This successful approach to acceleration of the rate of cycloaddition reactions after in vitro optimisation of ribozymes was recently reviewed elsewhere.[13]

The last few years have shown that nature is, indeed, able to use enzyme-catalysed pericyclic reactions, and these are, therefore, no longer to be regarded as an exclusive domain of synthetic organic chemistry. Mechanisms of catalysis, as well as the search for new Diels – Alderases, will without doubt be the subjects of future stimulating work.

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