The biosynthesis of polyketide-derived polycyclic ethers†

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The biosynthetic pathways to polyketide-derived polycyclic ethers, in bacteria, plants and marine organisms, have, until now, tended to be considered separately. The purpose of this article is to provide an integrated review of the common mechanistic aspects of polyether biosynthesis from these diverse sources. In particular, the focus will be on the proposed mechanisms of oxidative cyclisation, as well as on the known differences in polyketide chain construction between the terrestrial and marine polyethers.

1 Introduction

The term polycyclic ether, or simply polyether, is generally limited to only two classes of highly bioactive natural products that contain one or more ether rings, ranging from 5 to 9-membered; these are the polyether ionophores and the marine polyether ladders. Here, however, this definition will be expanded to include a group of similar compounds derived exclusively from a specific family of plants, the annonaceous acetogenins. The biogenesis of the polyethers has been the subject of an enormous amount of interest for over 30 years, and each of these types of metabolites reveals important facets of Nature's approach to the construction of ether rings.

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† Dedicated to Dr. Jonathan B. Spencer 1960–2008.

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Andrew obtained his Master of Chemistry degree at the University of Liverpool (1998–2002), where he acquired a keen interest in natural product biosynthesis. In 2002, he moved to Cambridge to pursue doctoral work under the supervision of Dr. Joe Spencer. His research focused on the genes governing oxidative cyclisation during monensin biosynthesis, as well as developing a general theoretical model for marine polyether construction. He currently works as a postdoctoral research associate in the lab of Prof. Peter Leadlay, where he studies the mechanistic details and evolution of polyether biosynthesis.

2 Fatty acid and polyketide biosynthesis

Both the terrestrial and the marine polyethers are derived from the classic polyketide biosynthetic pathway. The family of polyketide natural products is vast, and displays remarkable structural diversity and biological activity. The polyketide pathway[1] represents an adaptation of the fatty acid biosynthetic pathway,[2] and one which affords the pathway its diverse natural products. Construction of a fatty acid chain is initiated by the condensation of an acetate 'starter unit' with a malonate 'extender unit'. Acetyl-CoA and malonyl-CoA are first converted into enzyme-bound thioesters. The acetyl-CoA starter unit is loaded onto a specific cysteine residue on the β-ketoacyl synthase (KS). Similarly, the malonyl-CoA extender unit, in a reaction catalysed by malonyl acetyl transferase (MAT), is loaded onto a thiol of the acyl carrier protein (ACP). The fundamental chain extension step is catalysed by the ketosynthase and is a Claisen-like condensation facilitated by decarboxylation of the malonyl-ACP; this reaction gives the acetoacetyl-ACP. Subsequently, the ketoester is reduced by a ketoreductase (KR), dehydrated by a dehydratase (DH) and, finally, reduced further by an enoyl reductase (ER) (Figs. 1 and 2) This set of reactions completes the first round of chain extension, after which the chain is transferred from the ACP onto the ketoreductase, freeing up the ACP for the loading of the next extender unit. The extension process is repeated, two carbon units at a time, until a specific chain length is obtained. At this point, the enzyme-bound thioester is released from the fatty acid synthase, by means of a thioesterase, to give the free fatty acid. The polyketides are constructed on large enzymes very similar to the fatty acid synthase, namely polyketide synthases (PKS),[3] and the cycle of chain extension is directly analogous. The crucial difference lies in the fact that the ketoester may be left unprocessed by an absent or inactive ketoreductase,

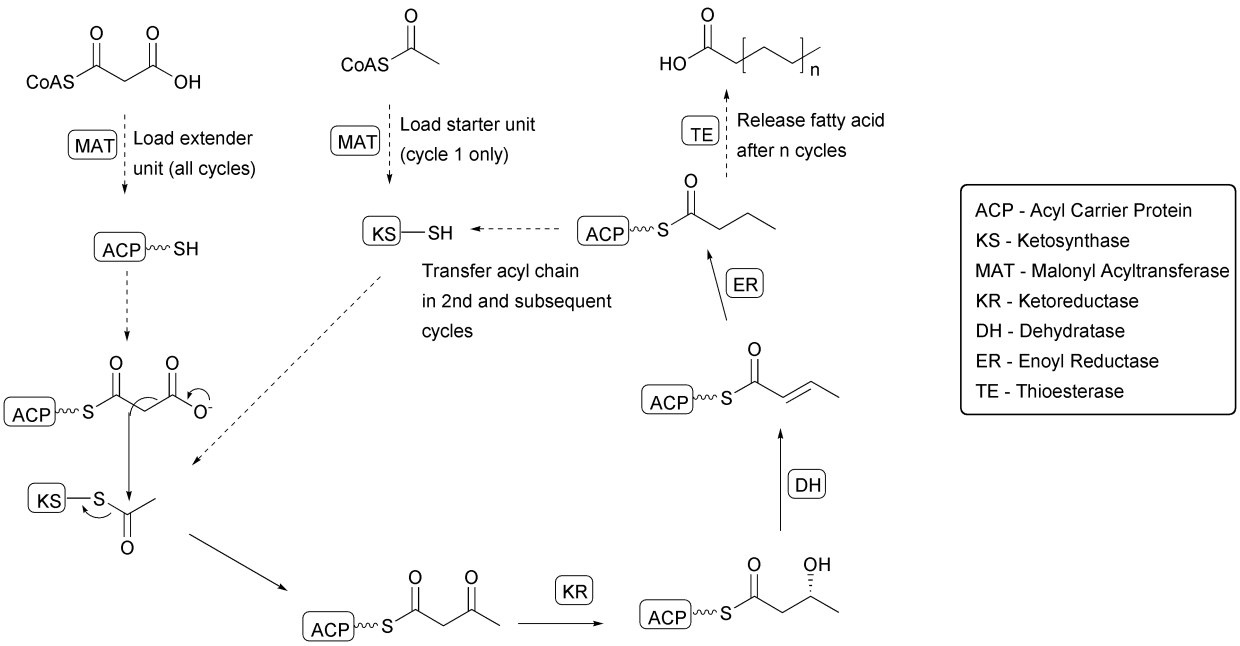


Fig. 1 Cycle of fatty acid biosynthesis.

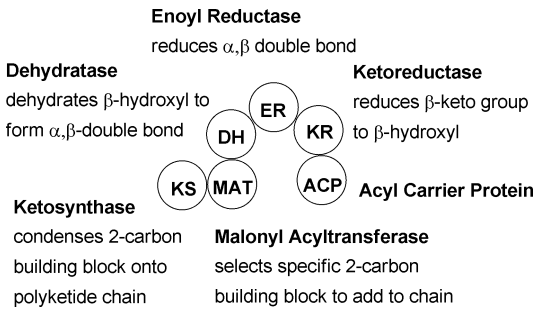


Fig. 2 Enzymes of the fatty acid synthase.

or reduced to varying degrees by the absence or presence of the subsequent catalytic steps. Thus, each extension cycle may afford either a keto-, hydroxyl, enoyl functionality or the fully saturated C_2-extension product. Structural diversity may be increased by the utilisation of alternative starter units or extender units other than malonate, in each cycle of chain extension (*e.g.* methylmalonate).[4,5] Further structural and functional modifications of this chain, whether during or after polyketide chain construction, afford the final natural product—the polyketide. It is these 'late stage' modifications that mould the distinctive structure of individual polyethers.

3 Polyether ionophores

Without exception, all known polyether ionophores are produced by actinomycetes. In particular, the vast majority are derived from the genus *Streptomyces* and, so far, over 120 such polyethers have been isolated and characterised.[6] The ionophore antibiotics share both structural characteristics and biological activities. From a structural perspective, all contain ether rings that are 5 or 6-membered and saturated; no larger ring sizes have been observed. Rings are either connected as a spiroketal system (2–3 rings—di- or tri-oxaspiro-cycloalkanes), or are separated by at least one single bond, with fused rings being absent from these structures. In addition, the polyether ionophores may contain a range of other structural elements, including hydroxy-, methoxy-, halo- and phenyl groups, and, often unusual heterocyclic systems, some of which may be important for biological activity. Typically, they exhibit a carboxyl group at one terminus and one or two hydroxyl groups at the other (Fig. 3).[7] By forming lipid-soluble, dynamically reversible, complexes with cations, they are able to transport them across biological membranes. This redistribution disrupts the carefully-controlled, energetically-demanding, balance of ions across bacterial membranes.[8] The polyether ionophore that has been most extensively studied is monensin, and it is thus appropriate that the majority of the research reported and discussed surrounding terrestrial polyether biosynthesis has centred on this particular ionophore. Monensin was first isolated and characterised in 1967 by Agtarap *et al.*,[9] and its polyketide nature was rapidly established; feeding of ^{14}C-labelled precursors demonstrated that the monensin backbone was constructed from five acetate, seven propionate and a single butyrate unit.[10] Further studies, utilising $^{18}O_2$, revealed that three of the ether ring oxygens and the terminal C26-hydroxyl were derived from molecular oxygen.[11,12] Prior to this, in 1974, Westley had proposed the formation of the terminal ether ring of a related ionophore, lasalocid, as resulting from the opening of an olefin-derived epoxide intermediate (Scheme 1).[13] This idea was specifically applied to the monensin structure in 1983 by Cane, Celmer and Westley (Scheme 2).[14] A linear triene intermediate was envisaged that is oxidised to form a triepoxide, which then undergoes a series of epoxide openings and ring closures to give the polycyclic structure. Assuming the epoxides were the result of a molecular oxygen-utilising mono-oxygenase, this scheme neatly accounts for the observed labelling pattern for $^{18}O_2$. This highly influential model has remained the

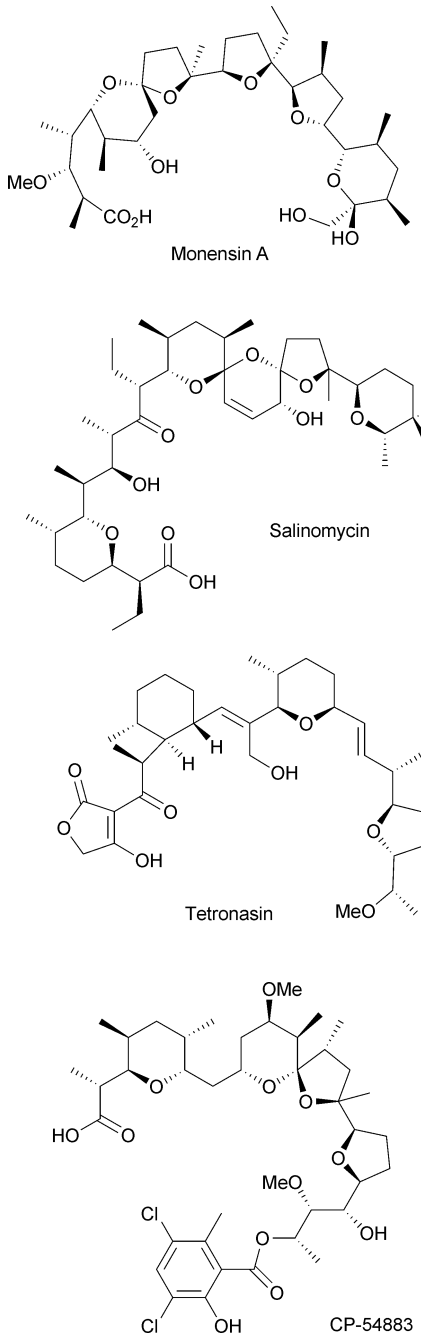
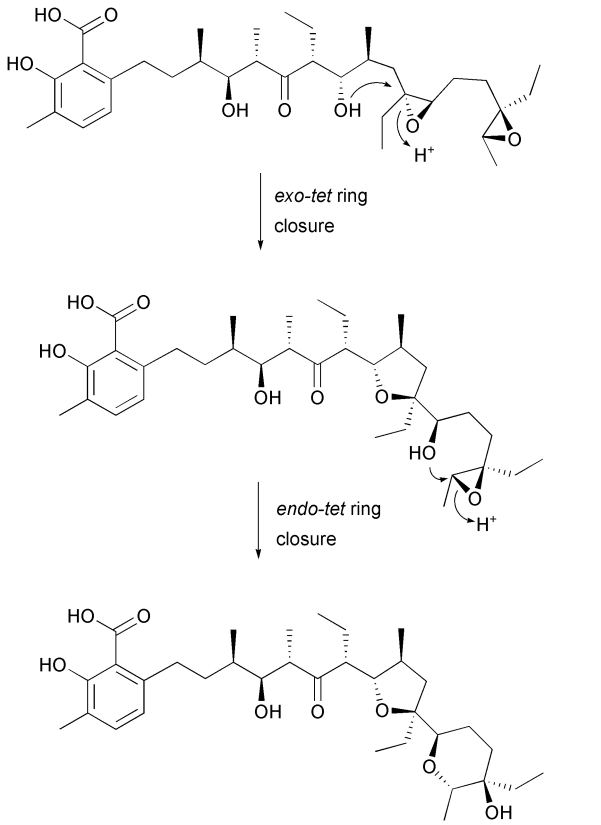
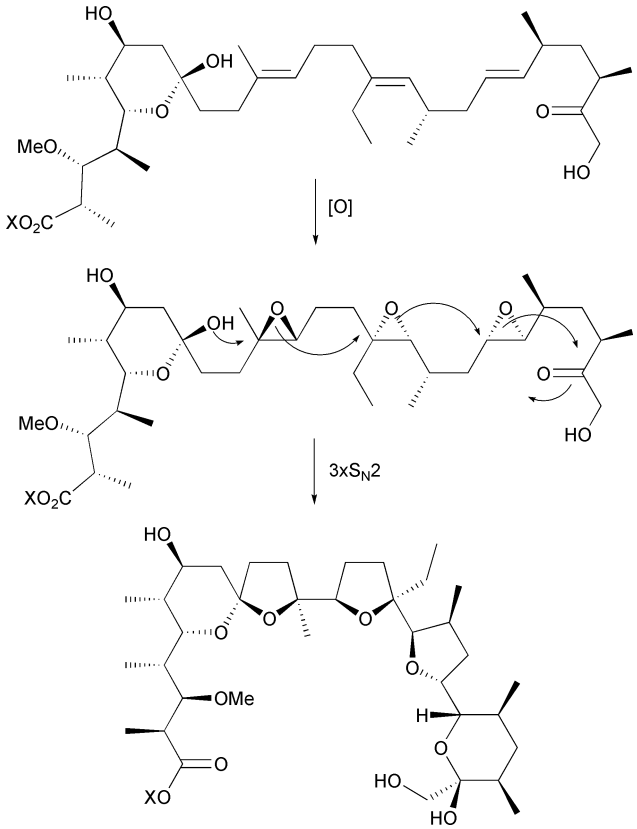


Fig. 3 Polyether ionophores.



Scheme 1 Proposed cyclisation of lasalocid.



Scheme 2 Cane-Celmer-Westley model for monensin biosynthesis.

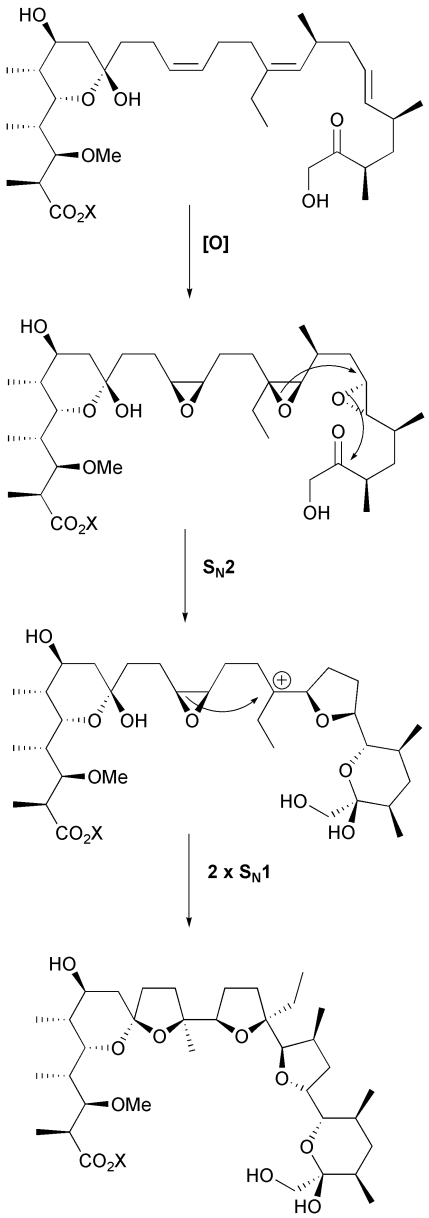
foremost up to the present, although variations on the theme have been proposed. An important feature of the Cane-Celmer-Westley (CCW) model is the requisite stereochemistry of the three double-bonds in the triene intermediate, which must all have a *trans* configuration. Two alternative models have been proposed, both of which involve a triepoxide intermediate with a different set of double-bond configurations.

The first of these was the Townsend-Basak model. This model invoked a series of [2 + 2] oxidative cyclisations utilising iron and necessitates a Z,Z,Z-triene.[15] Later, Staunton and Leadlay (Scheme 3) proposed a modified version of the CCW model that addressed possible concerns over the mechanistic aspects of the

CCW model. The CCW cyclisation mechanism involves two successive S_N2 inversions at tertiary centres, normally expected to be highly chemically disfavoured. The modified version overcame this concern by invoking S_N1 attack at these centres with retention of stereochemistry. Whereas the CCW mechanism is a cascade initiated by the S_N2 opening of the first epoxide, this alternative mechanism is initiated from the opposite end. In what appears to be a concerted (or almost so) process, the disubstituted epoxide is opened (S_N2 with inversion) by the second trisubstituted epoxide acting as a nucleophile as it opens to form a stabilised tertiary carbocation. This process could be assisted by activation of the terminal carbonyl, which would help activate the disubstituted epoxide towards the S_N2 reaction. The resulting carbocation is then quenched, with retention, by the opening of the first trisubstituted epoxide to yield a second tertiary carbocation, which is finally quenched by the hydroxyl. Overall, and distinct from the CCW mechanism, this proposal requires two trisubstituted *cis* epoxides and thus a Z,Z,E-triene precursor. As each of these models required a different set of double-bond configurations in the precursor, it was clear that if the triene intermediate could be detected and its stereochemistry determined, it would be helpful in distinguishing between these mechanistic possibilities.

The monensin gene cluster was fully characterised by sequence analysis of cosmid fragments from a monensin-producing strain of *S. cinnamonensis*.[16] The polyketide synthase is organised into twelve modules, each responsible for one cycle of chain extension, as would be predicted from the structure of monensin. The presence was noted of three novel genes not previously found in any complex polyketide gene cluster: *monBI, monBII*, and *monCI*. MonBI and MonBII display a significant polypeptide sequence homology to known ketosteroid isomerase[17] enzymes from *Comononas testeroni* and *Pseudomonas putida*.[18] If indeed the *monB* genes did encode isomerase-type enzymes, their presence might lend support to the Staunton-Leadlay cyclisation model. As each α,β-unsaturated double-bond of the growing polyketide chain is formed, this transiently activated bond might be isomerised by a MonB enzyme, *via* an extended enolate. If each of the trisubstituted double-bonds were isomerised from a *trans* configuration to *cis*, the result would be to convert an all *trans* (i.e. E,E,E) triene to a Z,Z,E configuration, as required by the above model. Further, if each 'isomerase' is responsible for only one double-bond, then the presence of two such enzymes is explained.

The *monCI* gene product shows considerable sequence homology to flavin epoxidases and thus it was inferred that this gene governs the epoxidation of the triene intermediate. Crucially, disruption of *monCI* led to the isolation and characterisation of a triene shunt metabolite, confirming its role as an epoxidase[19] and providing support for the notion that a single epoxidase suffices for oxidation of all three double bonds. Significantly, the three double bonds were each assigned a *trans* geometry (Fig. 4), strongly supporting the original CCW cyclisation model over both the Townsend-Basak and the Staunton-Leadlay proposals. This result unequivocally endorses the idea that oxidation and cyclisation from a linear polyene precursor is a viable biosynthetic methodology, with implications for the construction of polyethers in general. Meanwhile, the role of the *monB* genes was also clarified by gene disruption experiments.



Scheme 3 Staunton-Leadlay monensin cyclisation model.

The MonB proteins belong to an expanding family of enzymes that utilise the same structural scaffold to catalyse diverse reactions using acid–base catalysis. These include the ketosteroid isomerases, scytalone dehydratase,[20] the nuclear transport

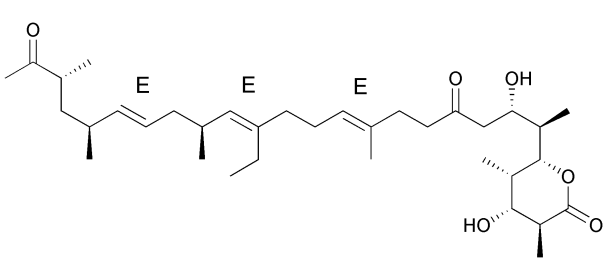
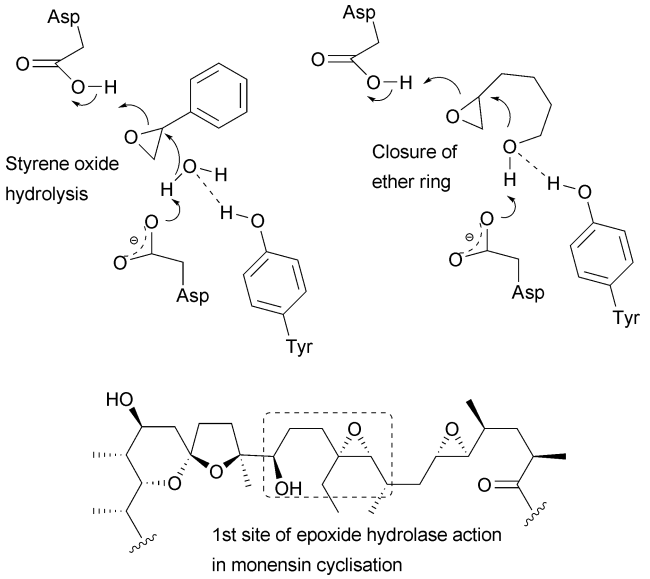


Fig. 4 Triene lactone isolated from *monCI*-null mutant of *S. cinnamonensis*.



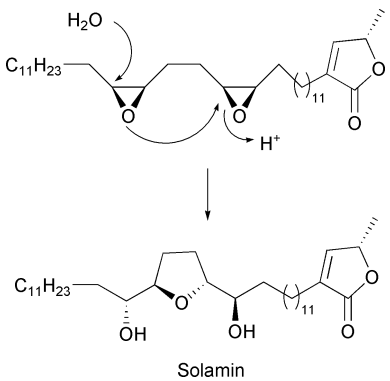
Scheme 4 Mechanism of limonene epoxide hydrolase at styrene oxide and proposed action in closing ether rings.

factors[21] and, most notably, limonene epoxide hydrolase.[22] A revised view was that the *monB* genes might encode hydrolases governing the cyclisation of the triepoxide intermediate (Scheme 4). This was borne out when, in the absence of the *monB* genes, *S. cinnamonensis* produced a range of partially cyclised intermediates, all of which could be converted to monensins by treatment with acid.[23] Other ionophore gene clusters, including nanchangmycin,[24] nigericin[25] and tetronomycin,[26] have subsequently been found to contain similar flavin-epoxidase and epoxide hydrolase genes that direct the oxidative cyclisation process.

4 The annonaceous acetogenins

The annonaceous acetogenins are a group of polyketide natural products isolated exclusively from plants and trees of the family Annonaceae.[27] The most common characteristic of these molecules is a C35 or C37 fatty acid chain terminating in a γ-lactone. Additional features may include olefin, hydroxyl, ketone or epoxide moieties, as well as tetrahydrofuran (THF) or tetrahydropyran (THP) rings (Fig. 5). To date, over 400 acetogenins have been isolated and characterised, but for the purposes of this discussion, only a few representative structures need be examined in detail. Although direct biosynthetic studies are lacking for these molecules, when considered together as a group, the structures of the acetogenins themselves strongly suggest their biosynthetic origin, with apparent biosynthetic intermediates being isolated alongside the end products. This is especially relevant to those acetogenins containing one or more ether rings. When considered alongside the analogous structures in the actinomycetes ionophores and the dinoflagellate ladders, it becomes evident that Nature's strategy for the construction of ether rings in the plant kingdom is common also to that of bacteria and the protists.

The solamins are a trio of isomeric acetogenins that possess the characteristic γ-lactone and a C35 chain in which a single THF ring is embedded. *trans*-Solamin (or simply 'solamin'), obtained from the roots of *Annona muricata*,[28] exists as a single diastereomer with a *trans* configuration across the ether ring. *cis*-Solamin, with a *cis* configuration across the ring, however, exists naturally as a mixture of two tetra-epimeric diastereomers, A and B.[29] All three are thought to derive from diepoxide intermediates, with cyclisation initiated by water (Scheme 5). The formation of two isomers of *cis*-solamin can be explained by contrasting mechanisms of cyclisation of a common diepoxide precursor—whether *cis*-solamin A or B is formed depends upon the direction of cyclisation, initiated by water attacking one or other of the epoxides (i.e. at either C_{15} or C_{20}) (Scheme 6). The presumed diepoxide precursor, diepomuricanin, is a known acetogenin isolated from the seeds of the same plant.[30] Although diepomuricanin hasn't been tested as an intermediate in the biosynthesis of the solamins, it seems reasonable to believe that they are correlated. Synthetic studies have shown that acid-treatment of diepomuricanin does indeed yield both diastereomeric forms of *cis*-solamin and (*trans*)-solamin.[31] Interestingly, these studies also showed that diepomuricanin A naturally exists as a pair of isomers with *syn*- and *anti*-configurations between the two epoxides, A1 and A2 respectively. Assuming an epoxide-opening cascade type mechanism, the *anti* form would be the precursor to the *cis*-solamins, with the *syn* isomer forming (*trans*)-solamin. Thus, the diene intermediate would need to be epoxidised from *opposite* faces to achieve the *anti* diepoxide, but the *same* face to achieve the *syn* (Scheme 6). One might naturally expect (*trans*)-solamin to have its own tetra-epimeric partner, as



Scheme 5 General mechanism of formation of the solamins *via* a diepoxide.

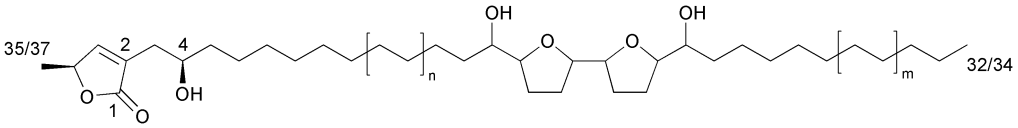
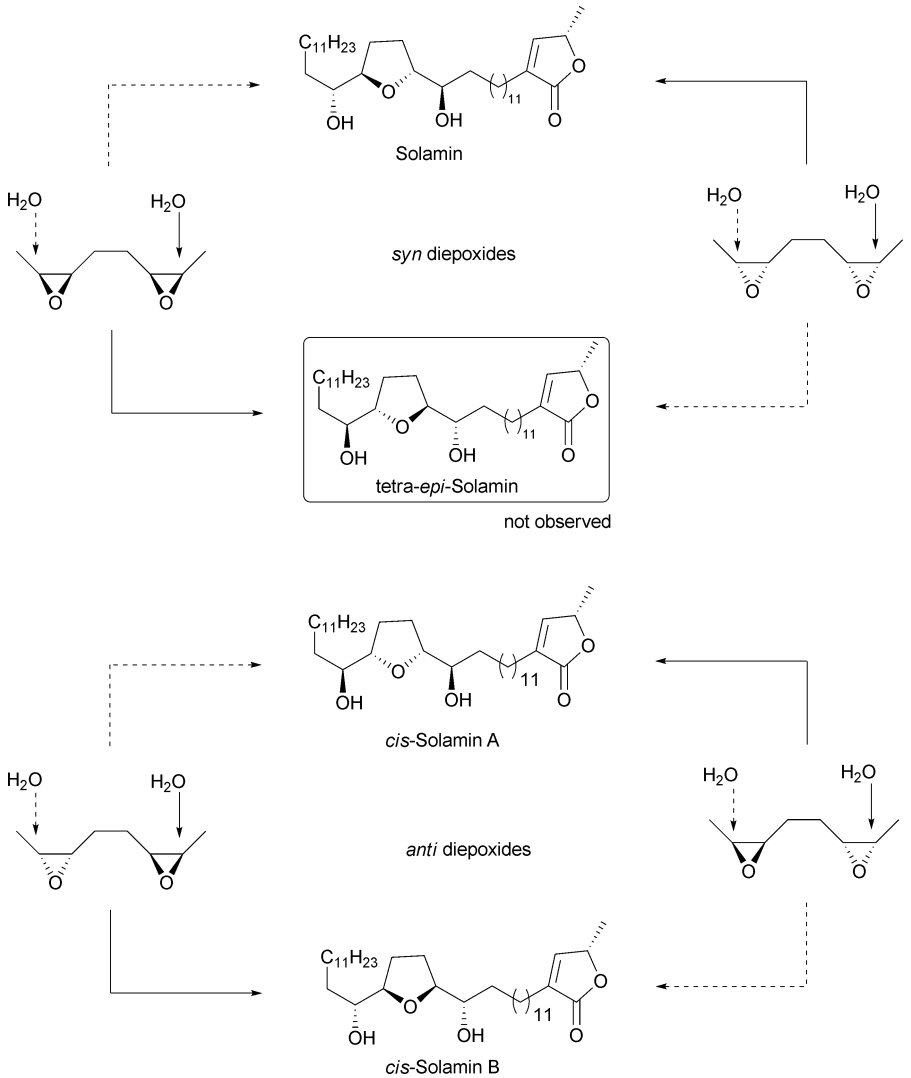


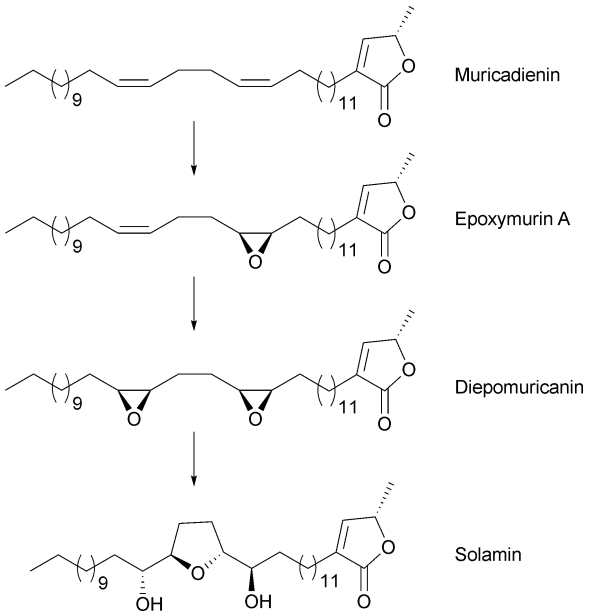
Fig. 5 General structure of polyether acetogenins.



Scheme 6 Modes of cyclisation to *trans*-solamin and *cis*-solamins A and B.

syn-diepomuricanin A can of course exist as two diastereomers and could theoretically cyclise in either direction (Scheme 6). The fact that this is not observed suggests, as is true with the poyether ionophores, that cyclisation is enzymatically controlled and not a spontaneous process. As well as the diepomuricanins, corresponding mono-epoxide precursor acetogenins have also been isolated from the *A. muricata*.[32] Epoxymurin A (Scheme 7) contains a single epoxide at C_{15-16} and a double bond at C_{19-20}, whereas epoxymurin B has the functionalities reversed, although the absolute stereochemistry of the epoxides hasn't been established. The hypothetical diene precursor to the epoxymurins is the latest 'intermediate' in the biosynthetic pathway to the solamins to have been isolated. Muricadienin-1, containing two *cis* double bonds, was purified from the roots of *A. muricata*, and is thought to be the common precursor to all three *cis* and *trans*-solamins[33] (Scheme 7). Overall, extracts of *A. muricata* have provided not only a 'polyether-type' acetogenin, but all of the hypothesised epoxide and olefin intermediates as well, from muricadienin-1 to the epoxymurins to diepomuricanin A to the solamins themselves. This strongly encourages the idea that plant-based polycyclic ethers, like their ionophore counterparts, are biosynthetically derived from polyenes *via* their corresponding polyepoxides.

Goniocin, isolated from the bark of *Goniothalamus giganteus*,[34] is the only known polyether acetogenin containing three ether rings. It is thought to be biosynthesised from a triepoxide intermediate (Scheme 8). Analogous to the solamins, the invoked intermediate is very closely related to a known acetogenin, tripoxyrollin, isolated from the seeds of *Rollinia membranacea*,[35] though not from those of *G. giganteus*. The epoxides of tripoxyrollin, however, are all shifted two carbons further along the chain than would be required to cyclise to goniocin, so it cannot be a true precursor to the latter. However, the presence of closely related metabolites in *G. giganteus*, appear to imply the role of a common triepoxide intermediate in their formation. Goniodenin[36] has a similar structure to goniocin, except that the third THF ring is replaced by a double-bond at C_{21-22}, in the position that would normally carry an epoxide in the hypothetical triepoxide intermediate (Fig. 6). Also, intriguingly, although the relative stereochemistries across the rings are the same as



Scheme 7 Biosynthetic route to solamin *via* known acetogenins.

goniocin, the absolute assignments are reversed. Thus, it seems that goniodenin results from the "incomplete" and opposite-face epoxidation of a common precursor triene. Synthetic studies, using an epoxidation and acid-cyclisation strategy, have also shown that goniodenin can be converted into a compound that is enantiomeric in the polyether region to goniocin.[37] An alternative fate of goniocin's triepoxide precursor is revealed by the structure of gigantecin, also from *G. giganteus*.[38] Instead of the purely intramolecular cyclisations that lead to goniocin, water apparently intercedes by attacking the central epoxide. The result is only two THF rings separated by a diol moiety[39](Scheme 9).

These examples demonstrate how plants generate a variety of structures by channelling common diene and triene intermediates along alternative oxidative biosynthetic pathways, in which comparison of intermediate and final structures reveal insights

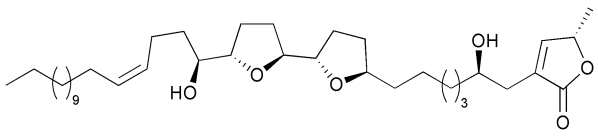
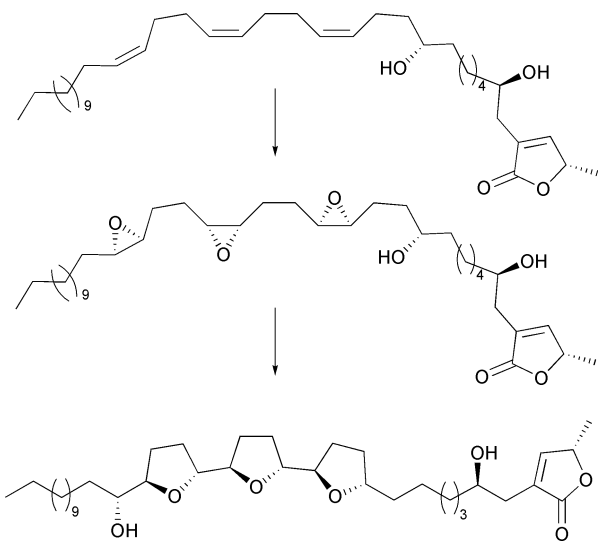


Fig. 6 Structure of goniodenin.

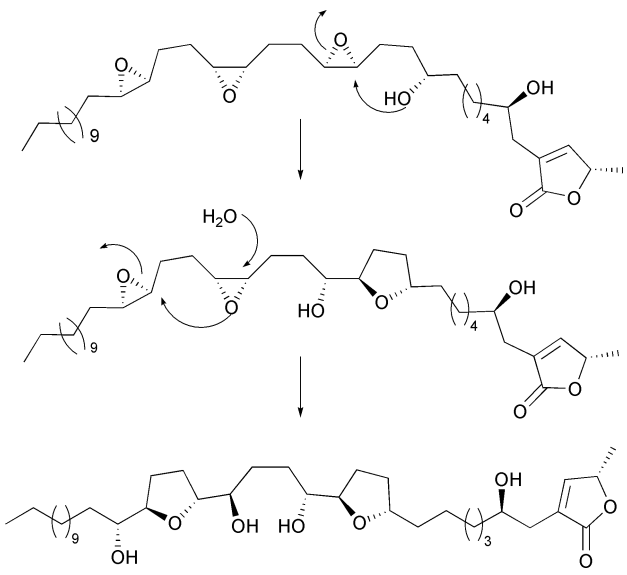
into their construction. Dissecting the chemistry of ionophore biosynthesis currently requires genetic manipulation of the producing bacteria in order to observe the otherwise transient intermediates, or at least shunted analogues of them. In contrast, the annonaceous plants naturally produce these 'intermediates' in detectable amounts.

5 Marine polyethers

Polyketide-derived marine polyethers are dichotomously distinct from the terrestrial, *Streptomyces* and plant-derived structures. All those characterised so far have a contiguous, fused ring system, with each oxygen constituting a single-atom bridge between rings. This gives them a characteristic ladder-like appearance, and they are generally referred to as ladder polyethers. Fourteen distinct ladder structures have been distinguished, falling into various structural classes (Fig. 7). The first of the ladder polyethers to be isolated were the brevetoxins, isolated from the dinoflagellate, *Karenia brevis*.[40,41] The brevetoxins have long been associated with the 'Red Tide' phenomenon, caused by the dense aggregation of a variety of toxin-producing unicellular phytoplankton, including *K. brevis*. This algal bloom causes a deep discolouration of the sea-water and poses a serious threat to aquatic ecosystems by killing a range of flora and fauna,[42] including humans who consume contaminated fish and other seafood. Other ladder polyethers include the ciguatoxins,[43–45] the yessotoxins,[46] gambieric acids,[47] gambierol[48] and the gymnocins.[49,50] Maitotoxin,[51,52] an extraordinary and lethal 3422 Da polyether, is both the largest and most toxic non-polymeric molecule known.



Scheme 8 Biosynthesis of goniocin *via* triene and triepoxide.



Scheme 9 Cyclisation of gigantecin.

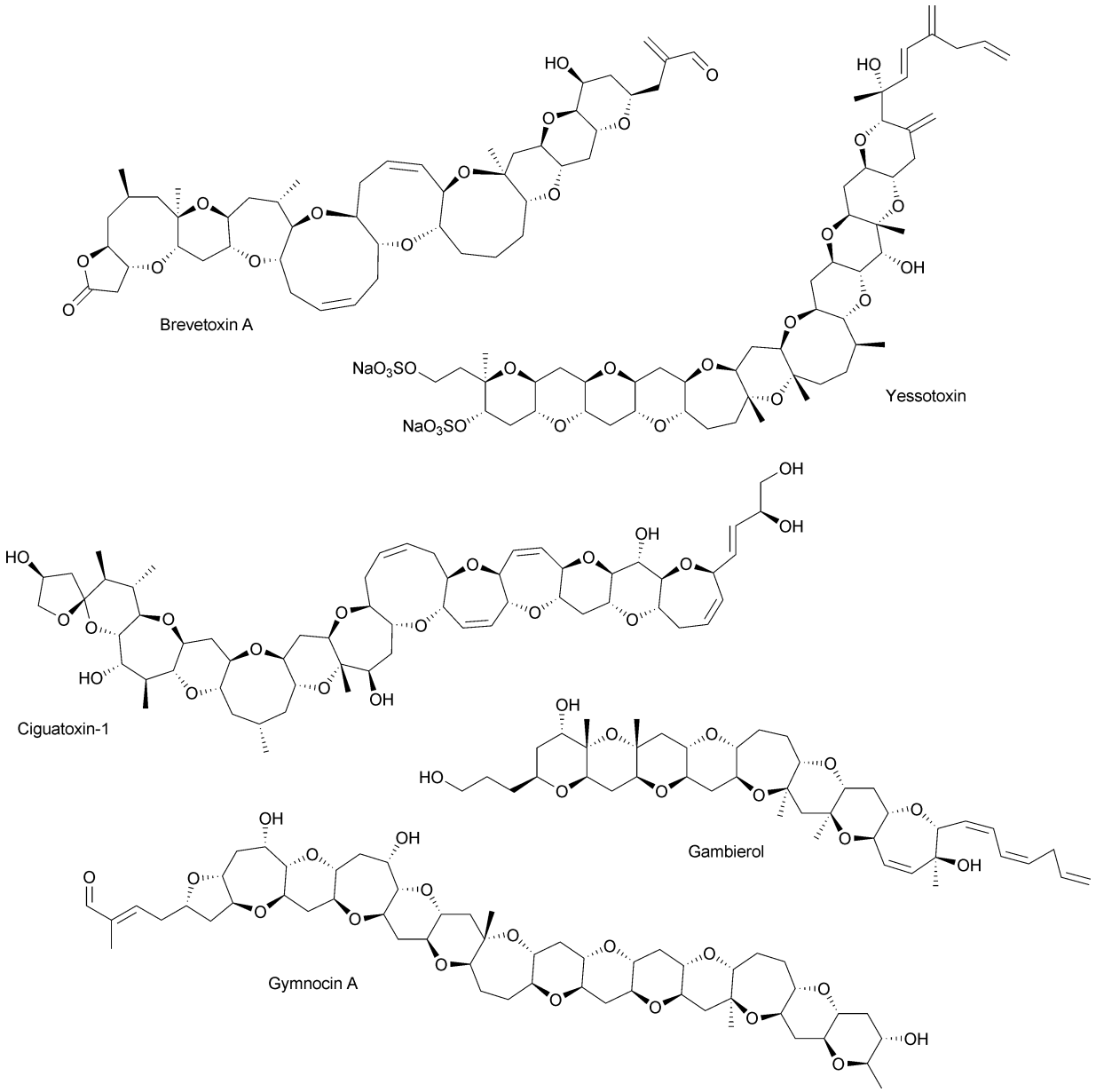
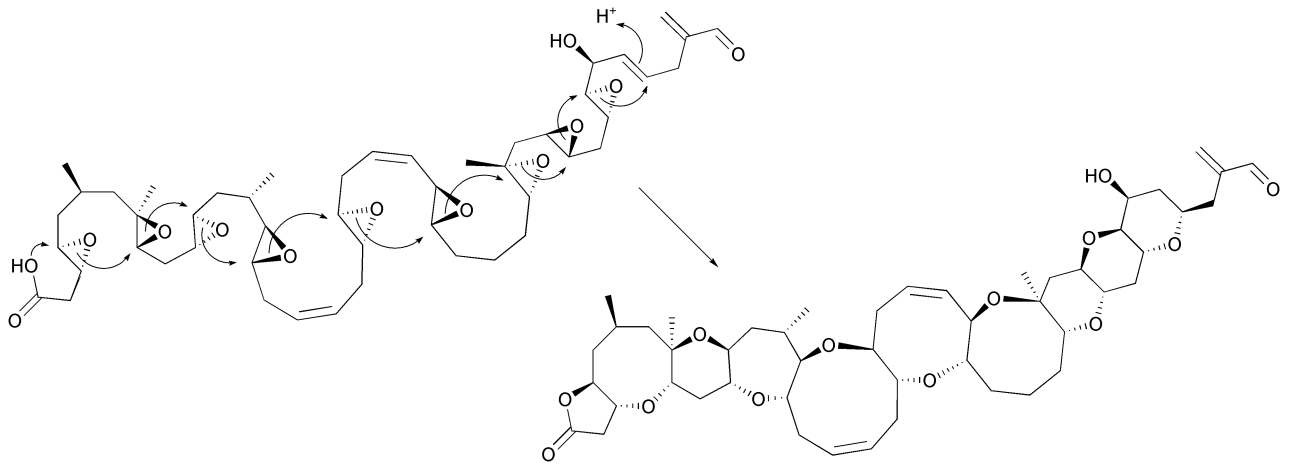
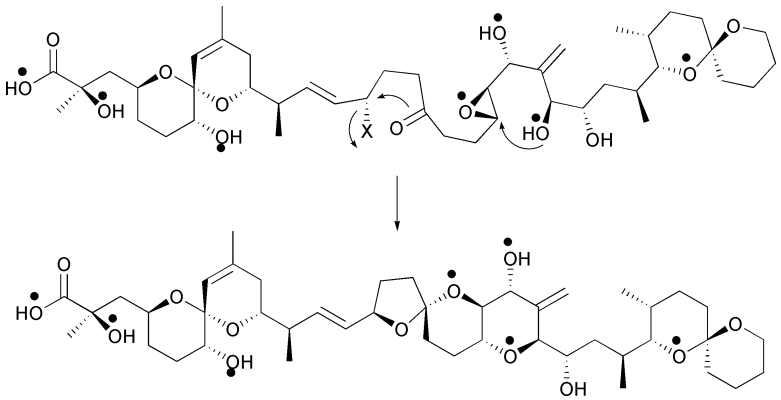


Fig. 7 Marine ladder polyethers.

The biosynthesis of the polyether ladders, whilst attracting speculation, has actually advanced little further than the identification of their polyketide origin. Although labelling studies have shed some light on the construction of the obligatory polyketide chain precursor,[53,54] anything further than this remains speculative. However, the model that has been proposed, and now validated, for monensin, was likely the inspiration for the most prominent model for the biosynthesis of the most well-known of the polyether ladders, brevetoxin. Both Shimizu and Nakanishi independently proposed this model—an octaepoxide precursor cyclises in a cascade of S_N2 epoxide openings, mechanistically similar to that initially proposed for monensin[55,56](Scheme 10). Indeed, the structures of all the marine polyether ladders suggest that cyclisation of a polyepoxide precursor might be a general biosynthetic strategy for their construction. Indirect evidence for such a mechanism is provided by the $^{18}O_2$-labelling pattern of okadaic acid, a related marine polyether toxin, suggesting an epoxide precursor to two fused ether rings (Scheme 11).[57,58] Also, the isolation of 27,28-epoxy-brevetoxin-B (double-bond in 8-membered ring epoxidised) may suggest the extraneous over-epoxidation of a polyene precursor,[59] although $^{18}O_2$-labelling studies are lacking. It remains largely in the realm of speculation as to whether the marine polyethers are derived from polyepoxides *via* polyenes, in a manner analogous to their terrestrial cousins. However, this general idea remains the most straightforward and satisfactory explanation. Further, across the entire range of known ladder polyethers, structural variation appears to be achieved through unusual biosynthetic manipulation of the carbon backbone (discussed later), whilst maintaining an invariant mechanism for



Scheme 10 Shimizu-Nakanishi brevetoxin cyclisation model.



Scheme 11 Proposed formation of okadaic acid fused ether rings from epoxide. Oxygens labelled with $^{18}O_2$ marked with dots.

ring formation. This is demonstrated by the *stereochemical uniformity rule*, as revealed by retrobiosynthetic analysis of all known ladder polyethers.[60] According to this rule, and assuming a given ladder polyether is derived from a polyepoxide precursor, all the epoxides must have identical absolute stereochemistries in order to achieve the observed stereochemical pattern in the final ring structure (Fig. 8). That all ladder polyethers adhere to this rule can be explained if a single mono-oxygenase is responsible for epoxidising the precursor polyene. The asymmetric epoxidation of each *trans* double-bond, in a consistent fashion by a single enzyme, on either the *Re* or the *Si* face, leads to a uniform polyepoxide consisting of either all-(*R,R*) or all-(*S,S*) *trans*-epoxides. Conversely, in order to achieve a non-uniform polyepoxide, each double-bond would either need to be differentially epoxidised by the mono-oxygenase, or, more likely, each double-bond would require its own distinct enzyme. Both scenarios, although possible, would greatly increase the complexity of the oxidation-cyclisation process. This model has prompted the re-examination and subsequent correction of the structure of at least one ladder polyether, brevenal. This is one of the newest members of the marine polyether family, isolated from *K. brevis*, and is also one of the smallest, containing only five contiguous rings. The structure initially proposed was largely in accordance with other ladder polyethers and appeared to derive from a polyepoxide in the usual way.[61] However, the terminal ring C26-hydroxyl was assigned a configuration that suggested the ring was formed by the opening of an epoxide with the opposite configuration than would be expected when comparing it to the rest of the structure. However, subsequent analysis resulted in the hydroxyl being reassigned with the opposite configuration,[62] as was predicted by the rule (Scheme 12). Similar arguments suggested that the structure of maitotoxin, the largest of the polyethers, might have been misassigned at a single ring junction, between the J and K rings. The structural elucidation of maitotoxin (Fig. 9), containing no less than 32 ether rings, was a Herculean task. However, as with the other, far smaller ladder polyethers, the ring system could be derived from a set of stereochemically identical epoxides, except for the J–K ring junction. This was anomalous in that it appeared to derive from an epoxide with opposite stereochemistry to the others (Scheme 13). This specific region of the molecule was acknowledged as particularly challenging to assign.[63] Prompted by these concerns, Nicolaou and his colleagues have synthesised large portions of the molecule to investigate this issue.[64–66] The results so far appear to be in agreement with the original assignment, but a crystal structure is needed to settle this matter definitively.

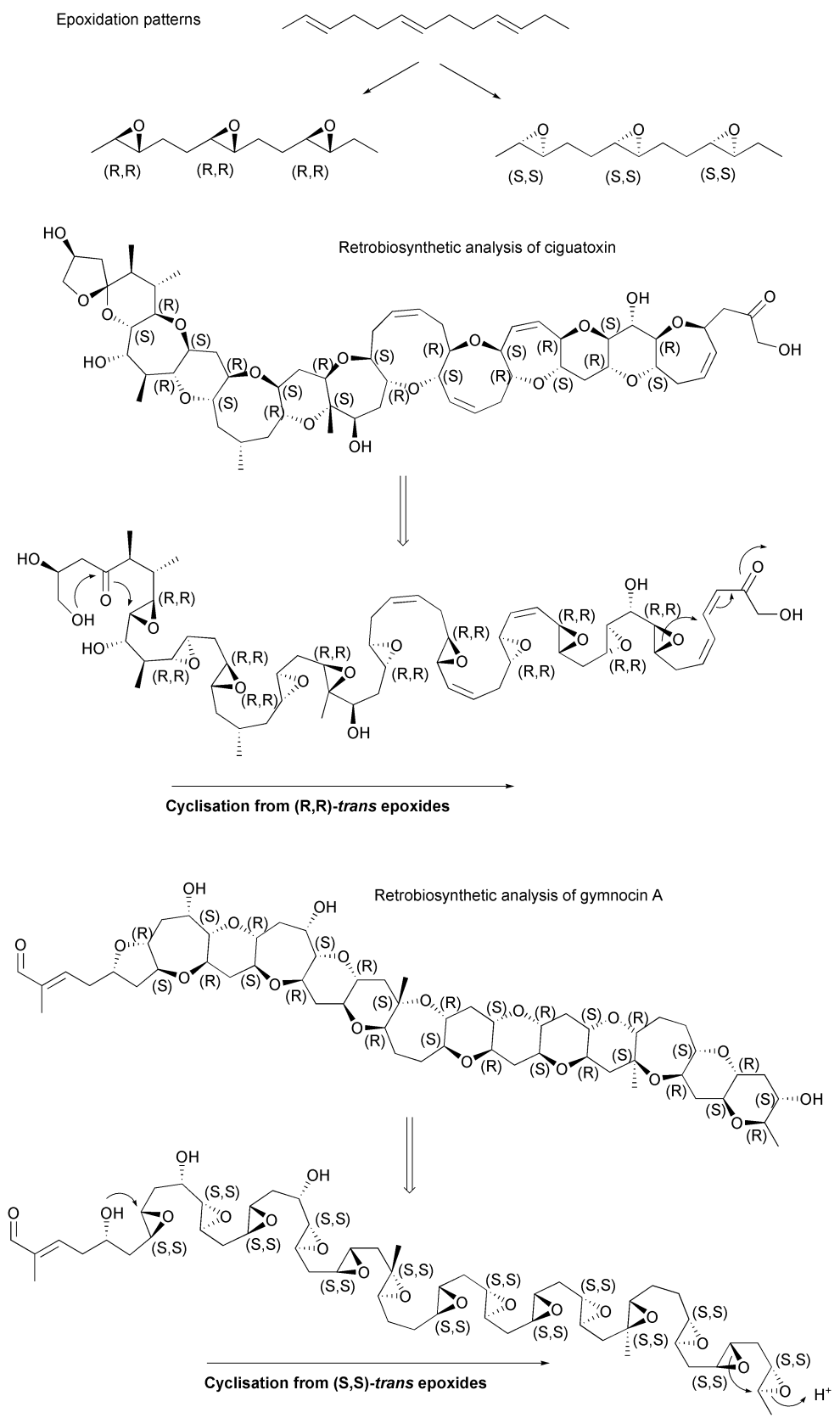
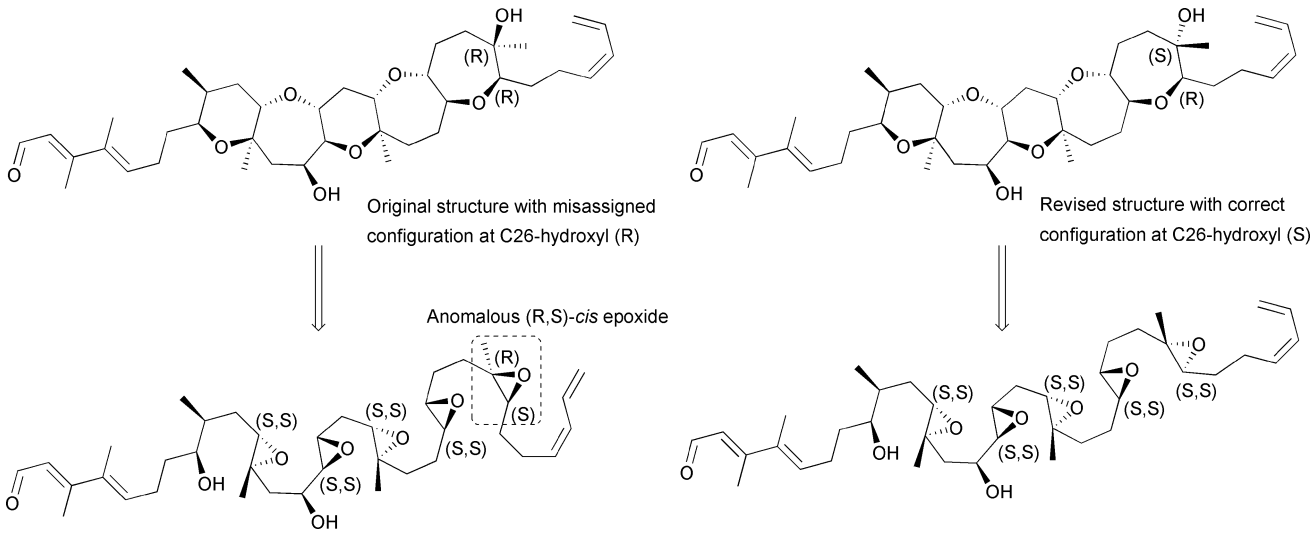


Fig. 8 Stereochemically uniform polyepoxides in ladder polyether biosynthesis.



Scheme 12 Retrobiosynthetic analysis of original and corrected structure of brevenal. The original structure would require an anomalous (*R*,*S*)-*cis*-epoxide in the precursor (boxed), whereas the corrected structure can be derived from a stereochemically uniform polyepoxide.

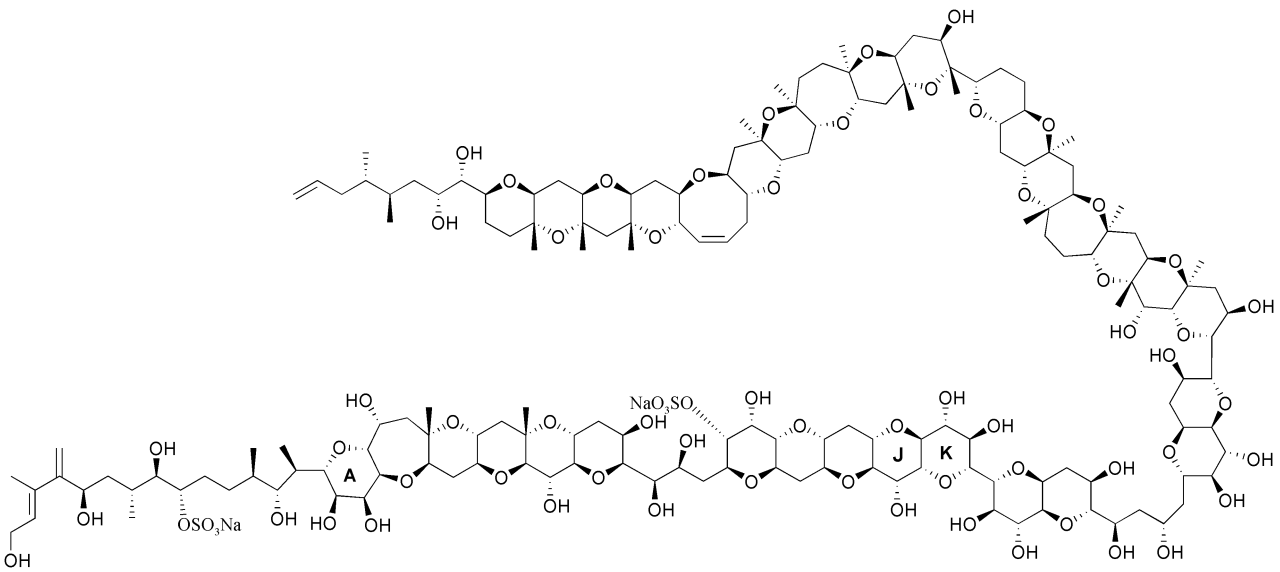
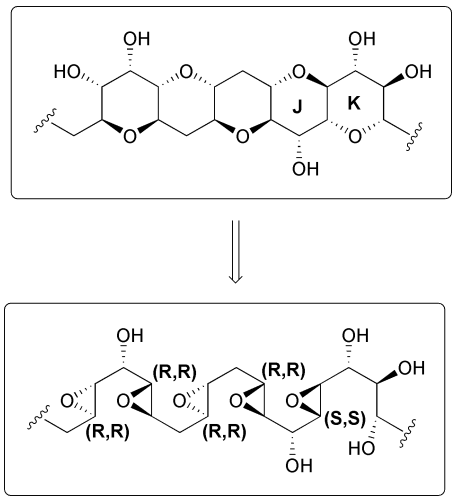


Fig. 9 Maitotoxin.

Although a polyepoxide intermediate may be feasible *en route* to the marine polyethers, a straightforward extrapolation of the Cane-Celmer-Westley cyclisation mechanism is not wholly satisfactory. Unlike the monensin triepoxide intermediate, which must cyclise in a series of energetically favoured *exo*-tet S_N2 closures, a *pre*-brevetoxin polyepoxide, for example, would undergo nine disfavoured *endo*-tet closures, each apparently violating Baldwin's rules[67] (Scheme 14). It is thus likely that an epoxide hydrolase-type enzyme would need to direct each ring closure. A possible precedent is given by lasalocid, a polyether ionophore from *Streptomyces lasaliensis*,[68] which bears a terminal ring that appears to derive from an *endo* epoxide ring opening. Further, the lasalocid gene cluster contains what is evidentially the first example of an *endo*-directing epoxide hydrolase.[69] Culture broths of *S. lasaliensis* also produce a small proportion (<1%) of the *exo*-product, isolasalocid, possibly the result of non-enzymatic catalysis of epoxide ring-opening.[70] It shouldn't be assumed, however, that Baldwin's Rules always dictate ring closure in the absence of directing enzymes. Recent model studies on polyepoxides have revealed that the detailed structure of the substrate and the nature of the solvent can profoundly influence the stereochemistry of ring closure.[71] By utilising a 'starter' ring as a template, or scaffold, for the subsequent epoxide openings, ring closure in water-containing solvents favoured formation of the fused, *endo*-derived, system over the *exo* alternative (Scheme 15). A combination of ring-strain effects and stabilisation by water molecules is thought to be responsible for this unusual, anti-Baldwin outcome; in the absence of water, Baldwin's Rules prevailed and the unfused *exo* rings were formed. Generally, in *dry* solvents, epoxide-opening



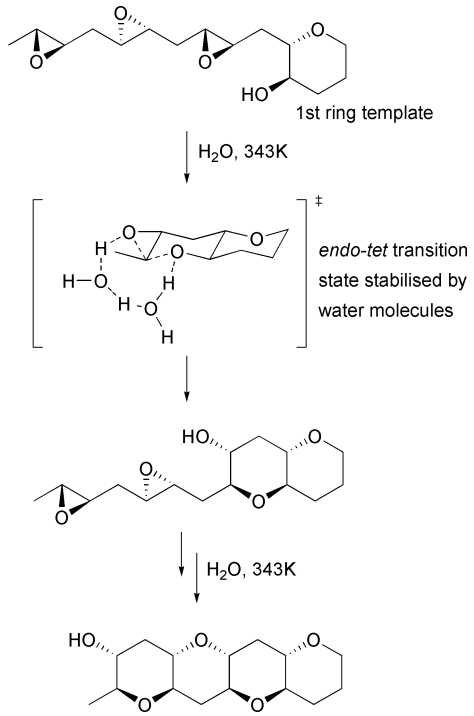
Scheme 13 Retrobiosynthetic analysis of GHIJK ring section of maitotoxin showing exceptional J/K ring junction from anomalous (*S*,*S*) epoxide.

cascades generated unfused *land* polyethers, whereas in *water* they generated fused *sea* polyethers. It thus can't be assumed that an *endo*-directing enzyme is being employed in ladder polyether cyclisation.

As an alternative to the *endo*-cyclisation of a polyepoxide, Giner has proposed that fused ether rings may be formed by the rearrangement of an epoxy ester[72](Scheme 16). Extrapolating this synthetic strategy to the biosynthesis of marine polyethers would require an all-*cis* polyene precursor. The formation of selectively positioned *cis* double-bonds in polyketide chains is certainly well precedented.[73]

6 Chain construction in polyether biosynthesis

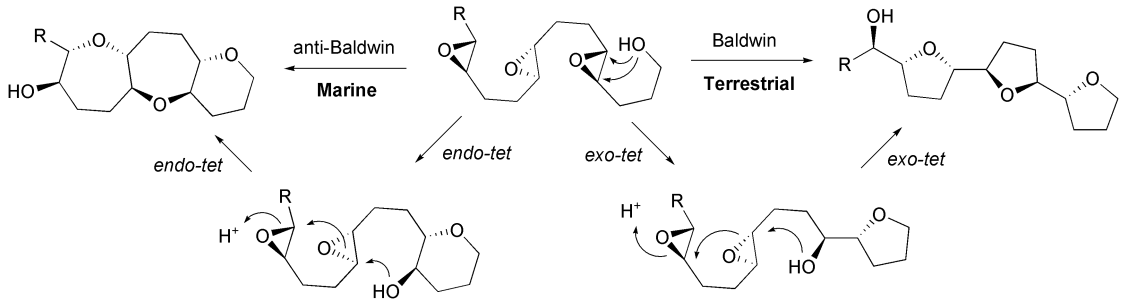
The polyethers from bacteria, plants and dinoflagellates, are clearly separated by the manner in which the carbon backbones are manufactured. As a rule, the biosynthetic sophistication of the backbone increases as one moves from plants to bacteria to marine protists, with dinoflagellates employing the most exotic chemistry. Essentially all annonaceous acetogenins are thought to derive from either C35 or C37 very long chain fatty acids (VLCFAs).[74] The γ-lactone is assumed to be constructed first; dehydrogenation then yields one or more *cis* double-bonds.[75] It is at this point that the acetogenin becomes a candidate for



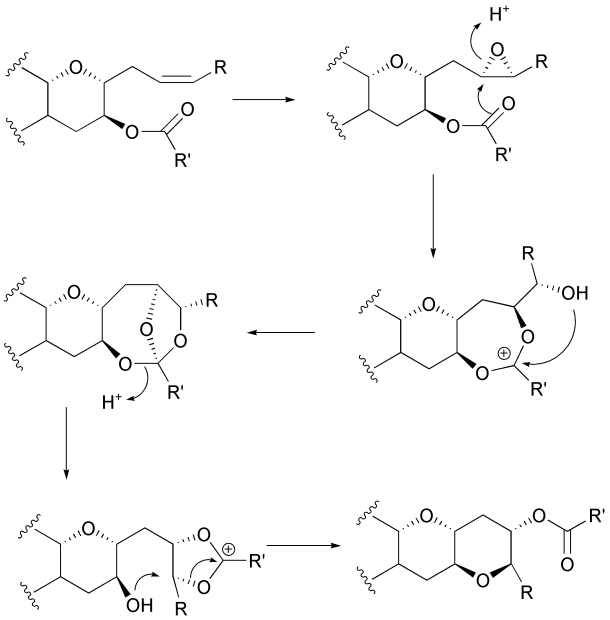
Scheme 15 Formation of a fused polyether from a polyepoxide catalysed by water.

transformation into a polyether, *via* the appropriate epoxy derivatives. Depending on the position and number of double-bonds and subsequent epoxidations, sites of hydroxylation and varying mechanisms of cyclisation, the range of polyethers produced from these templates is potentially very large. Many structures containing both THF and THP rings have already been characterised,[76] often in more than one diastereomeric form. As the field of annonaceous acetogenins is a relatively young one, we can assume many structures remain to be discovered.

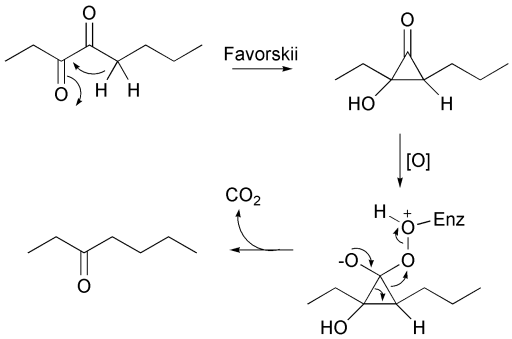
Similarly, over 120 natural polyether ionophores are known. Unlike the plant polyethers, as well as employing olefin-derived epoxides to construct ether rings, the ionophores commonly contain rings formed from carbonyl groups; these usually appear either as single-ring hemiacetals or as spiroketals with two or three rings joined. Monensin, for example, in addition to the rings derived from epoxides, contains a 5- and a 6-membered ring, joined as a spiroketal, as well as a 6-membered ring in the



Scheme 14 Alternative modes of cyclisation of a polyepoxide, leading to fused or unfused polycyclic ether.



Scheme 16 Formation of a fused polycyclic ether *via* an epoxy-ester rearrangement.



Scheme 17 Single carbon excision *via* a Favorskii-type reaction.

form of a hemiacetal. Salinomycin[77] (Fig. 3) has a dispiroketal system, comprising three connected rings, as well as an anomalous ring that appears to derive from neither epoxide nor carbonyl. The latter type of ring is also a feature of several of the marine polyethers, where the mechanism of closure of a terminal ring is unclear from the structure. Aureothin is an antifungal polyketide, from *Streptomyces thioluteus*, that contains a single THF ring that is constructed by an unusual bifunctional P450 mono-oxygenase without the assistance of carbonyl or epoxide functionality.[78] Some anomalous rings in polyethers might be made in a similar manner. Barring these exceptions, and unlike the acetogenins, ionophore biosynthesis utilises the normal functionalities developed on the polyketide synthase (PKS) in order to create ether rings—unreduced carbonyls, olefins and hydroxyls. One might reasonably suppose that the ancestors of the ionophores were the more straightforward, linear polyenes. It is thus tempting to suggest the ionophore biosynthetic pathways diverged from those of the polyenes *via* the acquisition of epoxidase genes. Although the oxidative cyclisation of a polyene to form a polyether is a terrific piece of 'post-PKS' engineering, it is the marine polyether producers that appear to utilise the most advanced and progressive of polyketide construction methodologies. Rather than being restricted to the [1,3] substitution pattern intrinsically built into the polyketide pathway, the carbon backbones of ladder polyethers, such as the brevetoxins and ciguatoxins, appear far more sophisticated. As a result, their biogenesis has proved much more difficult to unravel through classical feeding studies.[79] Feeding exogenous substrates to dinoflagellates is notoriously difficult, as they are often rejected, resulting in very low levels of incorporation. This problem is compounded by the low production levels of polyketide metabolites. In spite of this, stable isotope feeding has been successful in establishing the polyketide origin of the brevetoxins, as well as related dinoflagellates metabolites, such as okadaic acid[80] and amphidinolide.[81] However, when singly-labelled [1-^{13}C] and [2-^{13}C] and doubly labelled [1,2-^{13}C] acetate was fed to *K. brevis*, unexpected patterns of incorporation were observed that cannot be explained by the usual C2 extension sequence typical of polyketides. A simple polyketide will display the incorporation pattern, '**m-c-m-c-m-c-m-c**', etc, where '**m**'is the methyl of acetate [2-^{13}C] and '**c**' is the carbonyl [1-^{13}C]. However, brevetoxin B exhibited numerous consecutive methyls, including six '**m-m**' moieties, one '**m-m-m**' and one '**m-m-m-m**'. Similarly, brevetoxin A was labelled with seven '**m-m**' moieties, one '**m-m-m**' and one '**m-m-m-m**' (Fig. 10). Further, although four of the pendant methyl groups were labelled with [methyl-^{13}C]-methionine, the other three were derived from the methyl group of acetate. This unprecedented labelling pattern was explained by the

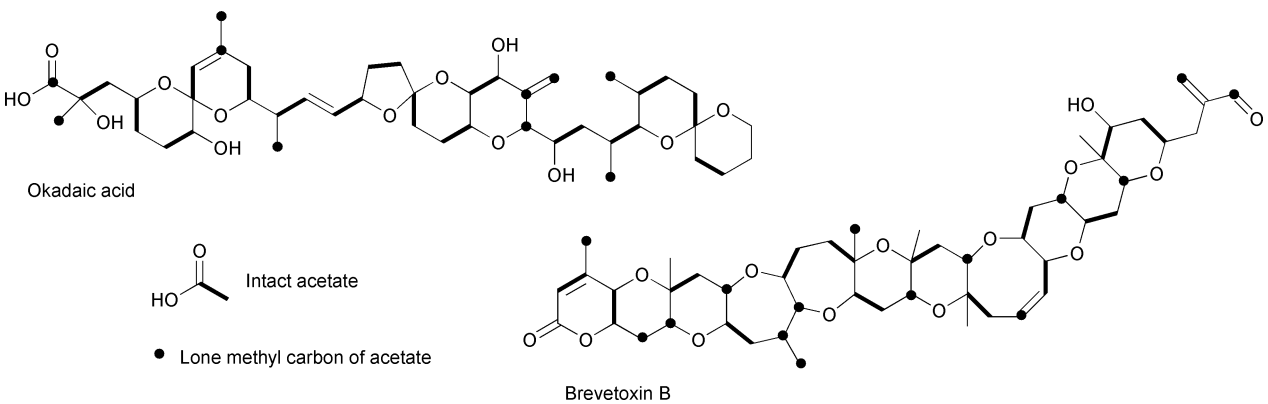
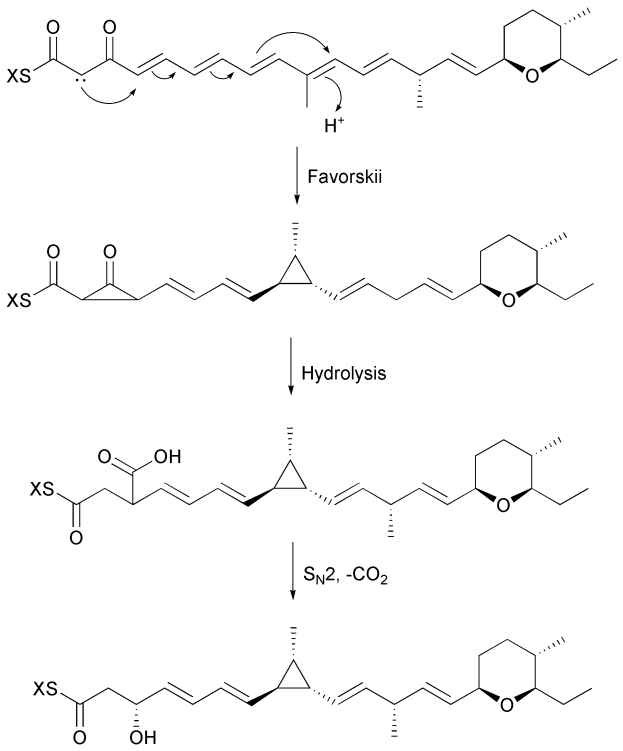


Fig. 10 Labelling pattern of okadaic acid and brevetoxin B.



Scheme 18 Formation of cyclopropane ring in ambruticin biosynthesis with concomitant excision of a single carbon.

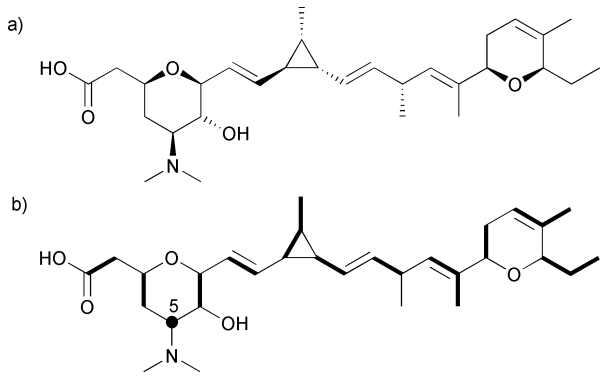


Fig. 11 a) Structure of ambruticin; b) Labelling pattern of ambruticin. Bold bonds indicate intact acetate/propionate units. C5 lone carbon indicated.

incorporation of intermediates from the tricarboxylic acid cycle.[82] However, subsequent feeding studies with the okadaic acid and dinophysistoxin (DTX) producer, *Prorocentrum lima*, suggest an alternative explanation.[83] The construction of the okadaic acid backbone mainly involves the usual successive incorporation of intact acetate units, but, at one point along the chain, this sequence is interrupted by a single carbon, '**m**', from the methyl group of acetate and, at another point, by two consecutive methyl carbons, '**m-m**' (Fig. 10). This was regarded as evidence, not of tricarboxylic acid cycle intermediates, but of an unusual carbon deletion process. Indeed, the uniform acetate enrichment observed suggested that unusual intermediates were not involved. If, during chain extension, a single carboxyl '**c**' carbon was excised before the chain was passed onto the next PKS module, the observed lone '**m**' carbons could be explained. The mechanism proposed involved a Favorskii-type rearrangement, following mono-oxygenase-mediated oxidation of the '**m**' carbon. This rearrangement forms a cyclopropanone intermediate that collapses with the aid of flavin-derived peroxide, releasing the carboxyl '**c**' carbon as carbon dioxide. Overall, this reaction yields a shortened chain with an oxidised methyl-derived '**m**' carbon (Scheme 17). This carbon-deletion process would not interrupt the flow of the polyketide extension process through detachment and reassembly of the nascent polyketide chain, or could even take place once the chain has been released from the PKS. Although this was a purely hypothetical proposal, a Favorskii-mediated carbon excision process has recently been shown to occur during the biosynthesis of the antifungal polyketide, ambruticin.[84] Ambruticin contains a cyclopropane ring that is thought to be formed on the growing polyketide chain with concomitant excision of a single carbon. The Favorskii reaction leads to closure of the cyclopropane ring through three conjugated double-bonds (Scheme 18). The cyclopropanone ring that is also formed is then hydrolysed and a single carbon released as carbon dioxide. The mechanism explains both formation of the cyclopropane ring and the unusual lone methyl '**m**' carbon (as in the brevetoxins and okadaic acid) (Fig. 11), although the enzymes involved have not yet been identified. Remarkably, however, an enzyme that performs a Favorskii-type reaction has been identified as involved in the biosynthesis of the marine bacteriostatic polyketide, enterocin, from *Streptomyces maritimus*. EncM is an FAD-dependent oxygenase that directs the rearrangement of the polyketide chain, through an oxidative Favorskii reaction, to achieve the unprecedented carbon skeleton of enterocin.[85] The Favorskii-type reactions occurring in ambruticin and enterocin biosynthesis offer clear precedents for ladder polyether construction, but more definitive insights must await progress in finding and defining the genes and enzymes of these remarkable biosynthetic pathways.

7 Acknowledgements

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