Exotic biomodification of fatty acids

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Many biotransformations of mid- to long chain fatty acyl derivatives are intrinsically interesting because of their high selectivity and novel mechanisms. These include one carbon transfer, hydration, isomerization, hydrogenation, ladderane and hydrocarbon formation, thiolation and various oxidative transformations such as epoxidation, hydroxylation and desaturation. In addition, hydroperoxidation of polyunsaturated fatty acids leads to a diverse array of bioactive compounds. The bioorganic aspects of selected reactions will be highlighted in this review; 210 references are cited.

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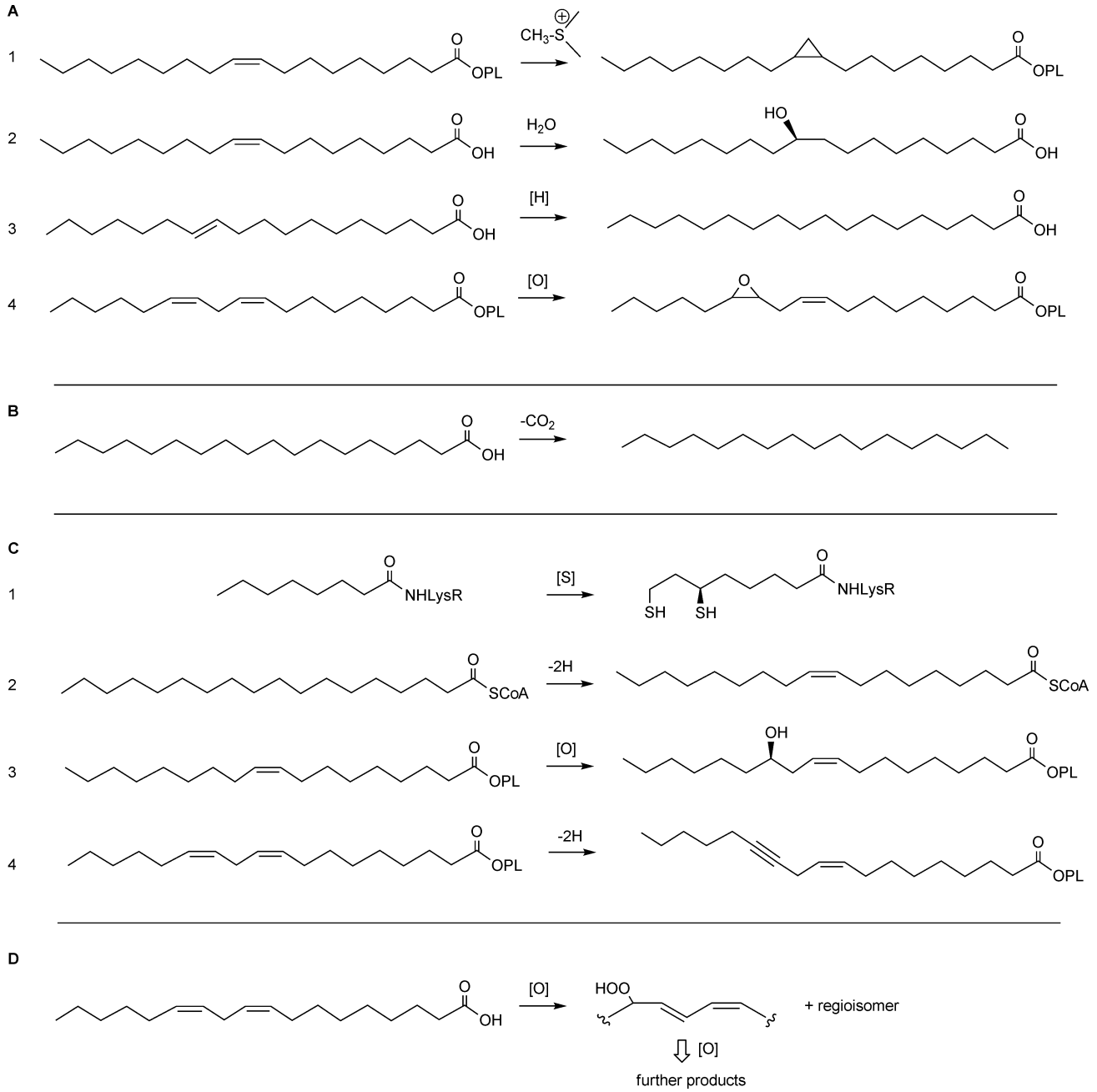
1 Introduction

Post-assembly modification of fatty acyl derivatives are mechanistically interesting biotransformations of importance to an emerging area of research known as lipodomics. These reactions can be broadly divided into four subsets (Scheme 1, ABCD): A) addition to unactivated double bonds including C-methylation, hydration, isomerization–hydrogenation or epoxidation, B) 1-carbon chain shortening at the carboxyl terminus, C) attack of unactivated C–H bonds resulting in thiolation, dehydrogenation (desaturation) or hydroxylation, D) further oxidative modification of polyunsaturated fatty acids *via* hydroperoxide intermediates. Many of these reactions are without good laboratory precedent and are thought to involve electron-deficient reaction intermediates such as carbon-centred radicals or carbocations. To elucidate how such highly reactive intermediates are generated in the hydrophobic interior of the enzyme active site and then directed to product with such exquisite selectivity is a worthy research objective. These efforts are particularly relevant to ongoing efforts directed at developing synthetically useful C–H activation chemistry.

Characterization of the lipodome relevant to various disease states has stimulated renewed activity in the study of fatty acid biomodifying reactions, first studied many years ago. Prominent examples include C-methylation[1] as it occurs in mycobacteria, the causative agents of tuberculosis and leprosy, stearoyl CoA Δ^9 desaturation, overexpression of which is implicated in the



Scheme 1 Various fatty acid biomodifications (PL = phospholipids, NHLysR = pyruvate dehydrogenase, CoA = coenzyme A).

"metabolic syndrome",[2] production of "natural" *trans* fatty acids during biohydrogenation[3] and hydroxylation–desaturation of sphingolipids as part of the apoptotic cascade.[4] Development of selective, mechanism-based inhibitors for these processes is of intrinsic interest and may be of significant therapeutic value.

A further stimulus for research in the bioorganic chemistry of lipid transformations has arisen from genomic research into various plant seed oils—a treasure trove of exotic functionality such as cyclopropene rings, chiral allenic units and polyacetylenes.[5,6] Exploiting the enzyme activity responsible for generating high-value compounds is a major objective of plant biotechnologists who envision using plants as green factories.[7] Included in this scenario is the production of hydrocarbon-based biofuels as a renewable energy source.[8] A thorough understanding of reaction mechanism is required to guide and interpret protein engineering experiments intended to tune enzyme chemoselectivity and give bioproducts the desired properties. A case in point is the engineering of Δ^{12} desaturases to function as highly enantioselective 12-hydroxylators (Scheme 1C, reaction 3).[9] Another exciting dimension to seed oil research stems from the fact that many phytolipids have interesting biocidal properties. For example, cyclopropene-containing fatty acids such as sterculic acid found in the seed oils of selected subtropical plants are potent inhibitors of mammalian Δ^9 desaturases.[10] Other topics of importance in the emerging area of chemical ecology involve the biosynthesis

of species-specific insect pheromones[11] and other signaling agents such as oxylipins.[12]

A third area of research where fatty acid biotransformations play a prominent role relates to the way many organisms adapt to environmental stress such as hypoxia (desaturase hyperexpression),[13] temperature extremes (desaturation–bio-hydrogenation),[14–16] low pH (cyclopropane formation)[17,18] or the presence of toxins (*cis–trans*-isomerization,[19] ladderane formation[20]). By inducing suitable reactions, the biophysical properties of structural lipid components can be adjusted to maintain optimal fluidity of membrane barriers—a condition that is critical to cellular function.

In this review, we discuss the mechanistic aspects of fatty acid biomodification with an emphasis on reactions not covered previously in a recent overview of desaturases.[21] Valuable background material is available from previous reviews of fatty acid biosynthesis,[22–24] sulfur insertion biochemistry,[25–28] bacterial lipids[17] and standard reference works on lipid biochemistry[29,30] and plant lipids.[31] Consultation of reviews in the sterol area is also recommended as many of the reactions discussed in this review have close parallels in sterol biosynthesis.[32,33] Indeed, mechanistic models and probes developed for fatty acid biomodification have been successfully applied to the study of sterol transformations[34,35] and *vice versa*.[36]

2 Olefin addition

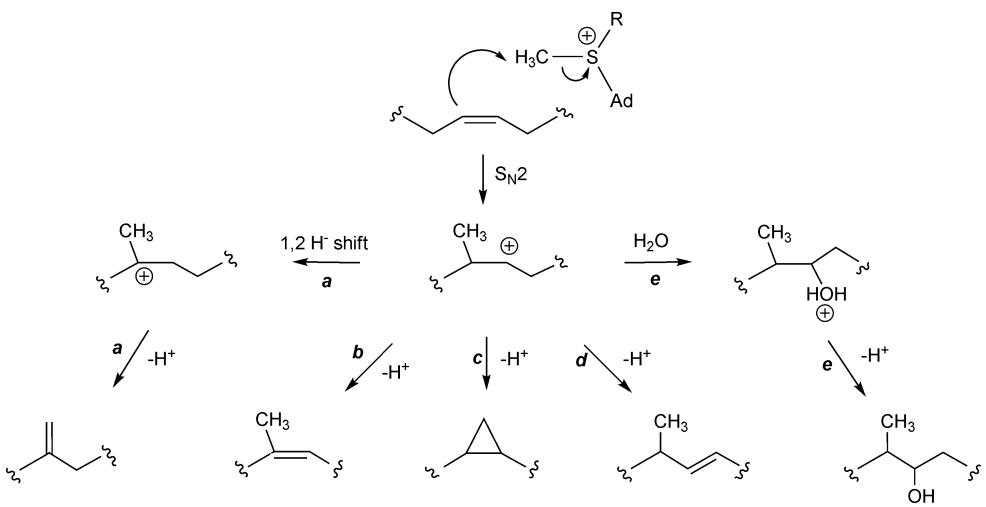
Cellular unsaturated fatty acyl derivatives are major components of cellular lipids and are typically formed through highly selective, O_2-dependent desaturation reactions (Scheme 1C, reaction 2).[21] In some microorganisms, olefinic fatty acids are formed anaerobically *via* a modified fatty acid synthase-catalyzed sequence.[29,30] The isolated carbon–carbon double bond in olefinic fatty acids is often modified by energetically "difficult" addition reactions as discussed in the following sections.

2.1 C-methylation reactions

A very common olefin addition reaction is *S*-adenosyl methionine (S.A.M.)-dependent C-methylation. The mechanism for this transformation was first proposed by Lederer and is thought to be initiated by methyl transfer from S.A.M. to form a carbocationic intermediate.[37] This species then collapses rapidly by 1,2-proton elimination (Scheme 2, pathways a,b,d) or 1,3-proton elimination (pathway c). Quenching of the putative intermediate with an enzyme-bound water molecule (Scheme 2, pathway e) is rarely seen but has been postulated to occur in the generation of the CH_2–CH(OMe)–$CH(CH_3)$–CH_2 moiety found in some mycolic acids.[38] The choice of reaction pathway is presumably determined by the specific location of a proton acceptor in the active site of each individual methyltransferase.

This mechanistic scheme has been difficult to model since unactivated alkenes are such poor nucleophiles. However, it has been shown that sulfonium salts with non-nucleophilic counter ions are able to alkylate alkenes to generate a variety of olefinic products by subsequent deprotonation.[39] The driving force for enzyme-catalyzed methyl transfer can be rationalized in terms of active site-induced separation of the sulfonium centre in S.A.M. from its counterion.[40] In addition, aromatic amino acid residues are thought to stabilize carbocationic intermediates in a hydrophobic pocket through π–cation interactions.[41] Attempts to probe the energetics of biochemically relevant classical and nonclassical carbocations have been carried out using increasingly sophisticated theoretical tools in combination with site-directed mutagenesis experiments.[42–44]

2.1.1 Cyclopropane synthase. The serendipitous discovery of cyclopropane ring-bearing fatty acids in lactic acid bacteria[45] by the Hofmann group was prompted by the observation that *Lactobacilli*-based assays of biotin were occasionally compromised by the growth-stimulating activity of unsaturated fatty acid contaminants.[46] A systematic investigation of other microbial lipids demonstrated that the occurrence of this unusual type of fatty acid was widespread and also extended to the seed oils of some subtropical plant species.[47] Some examples of naturally occurring cyclopropyl fatty acid derivatives are shown in Fig. 1. These include lactobacillic acid—the ubiquitous bacterial monocyclopropyl fatty acid, U-106305—a bizarre, oligocyclopropanated antifungal metabolite found in a *Streptomyces* strain[48]



Scheme 2 Possible products derived from *S*-adenosylmethionine-dependent C-methylation of an unactivated olefin.

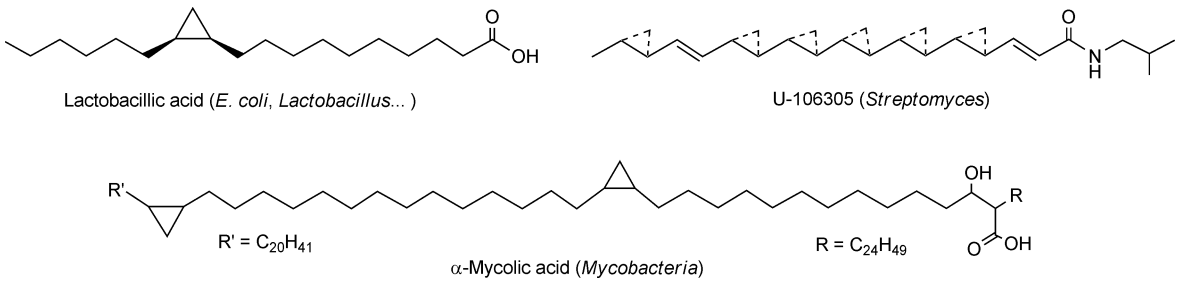
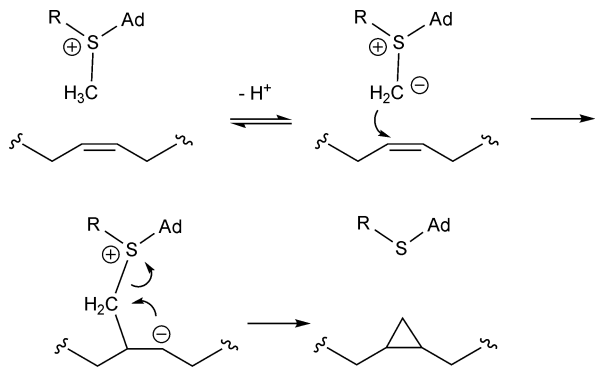


Fig. 1 Some naturally occurring cyclopropyl fatty acids.

and the very long chain dicyclopropanated mycolic acids found in *Mycobacteria*.[1]

In plants, cyclopropyl fatty acids would appear to serve as intermediates *en route* to the corresponding cyclopropene-containing fatty acids although this remains to be proven unambiguously.[49] The biological role of the alkene–cyclopropane conversion in microorganisms is not intuitively obvious and various proposals have been advanced.[17] The impact of "cyclopropanation" on the Tc (temperature of gel to liquid crystalline phase transition) of model unsaturated lipids is relatively modest.[50,51] The ability of some microorganisms to tolerate environmental stress such as low pH has been attributed to the conversion of unsaturated fatty acids to their cyclopropyl counterparts.[17,52] This observation has been extended to *Helicobacter pylori*, the microorganism responsible for the development of gastritis and peptic ulcer disease.[53] The ability of this microbe to withstand the harshly acidic environment of the stomach may be due, in part, to its cyclopropyl lipid content.[54] Another medically related observation, namely that the pathogenicity of TB-causing mycobacteria can be correlated with cyclopropane content of the mycolic acid-containing cell wall, has driven much of the renewed interest in the enzymology of cyclopropane ring formation.[55–57]

The current consensus mechanism for S.A.M.-dependent cyclopropane ring formation is that proposed initially by Lederer (Scheme 2, pathway c).[37] Several lines of evidence now support the operation of this mechanism rather than a pathway involving a sulfur ylid (Scheme 3) or metal carbenoid formation (not shown).



Scheme 3 The sulfur ylid pathway for biological cyclopropane ring formation.

1. Crystal structures of three soluble cyclopropane synthases involved in mycolic acid biosynthesis have been published and reveal the presence of a typical S.A.M. binding motif proximal to a hydrophobic pocket that can accommodate a fatty acyl hydrocarbon chain.[57] A catalytically essential bicarbonate ion in the active site has been identified and it has been suggested that this species is responsible for deprotonation of the putative carbocationic intermediate formed after methyl transfer.[58,59] Importantly, the absence of a metal or metal binding site in these structures effectively eliminates the possibility of a sulfur ylid-derived, metal carbenoid mechanism for biomethylenation.[60] In addition, a search for metal ions in purified cyclopropane synthase isolated from *E. coli* was carried out using inductively-coupled plasma (ICP) spectroscopy; no evidence for the presence of catalytically active metal ions was found.[61,62]

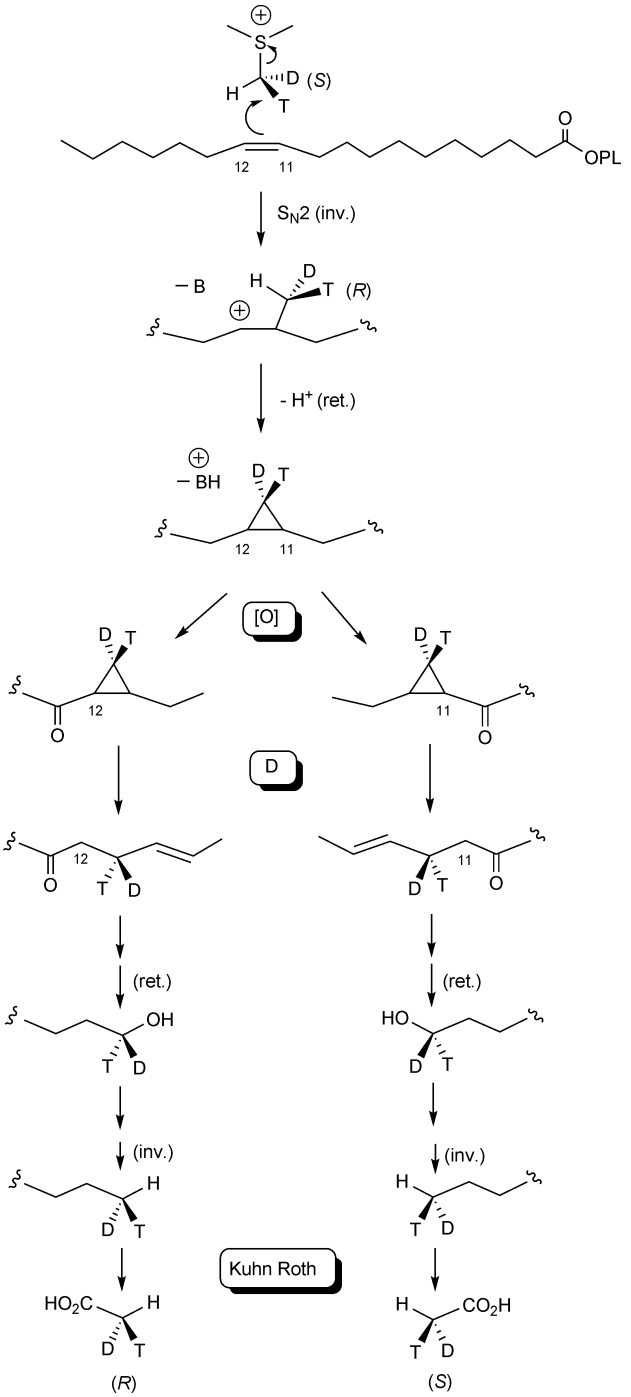
2. *In vitro* kinetic isotope effect studies using purified *E. coli* cyclopropane synthase showed that while the methyl transfer step is kinetically important, the deprotonation step is clearly not, as might be anticipated for collapse of a carbocation.[61] The value of the secondary inverse deuterium isotope effect using a trideuteromethyl S.A.M. ($\sim$ 0.87) is typical of a tight S_N2 transition state for methyl transfer.[63] These results corroborate earlier measurements of primary deuterium kinetic isotope effects using an *in vivo L. plantarum* cyclopropanating system where labeled S.A.M. was biosynthesized from methionine methyl isotopomers (RS-CD_3, RSCD_2H and RSCDH_2).[64] In this work, an intramolecular or "intrinsic" primary deuterium KIE ($k_H/k_D \sim 3$) was observed for cyclopropyl ring formation when mono- and dideuteromethyl methionine were employed, however, this KIE was completely masked when an intermolecular, competition experimental design (RS-CD_3 *vs.* RS-CH_3) was utilized. This result indicated that the deprotonation step is not a rate-limiting step.[65]

3. When a series of S.A.M chalcogen analogues were used as substrates for cyclopropane synthase, the observed trend in kinetic parameters was consistent with a methyl transfer mechanism rather than sulfur ylid formation.[66]

4. Vinylically fluorinated oleate substrate analogues were not accepted as substrates for cyclopropane synthase in contrast to what might be expected for attack by a sulfur ylid species.[67]

5. Thiastearate substrate analogues inhibited cyclopropane ring formation *in vivo*, possibly by methyl transfer from S.A.M. to form a "sticky" methyl sulfonium species that mimics the carbocationic intermediate.[68]

6. Lactobacillic acid biosynthesis as it occurs in *L. plantarum* was studied by the Arigoni group (E.T.H. Zürich) using chiral methyl group methodology (Scheme 4). Degradation of the multiply-labelled product derived from an (*S*)-chiral methylmethionine, followed by stereochemical analysis of the resultant



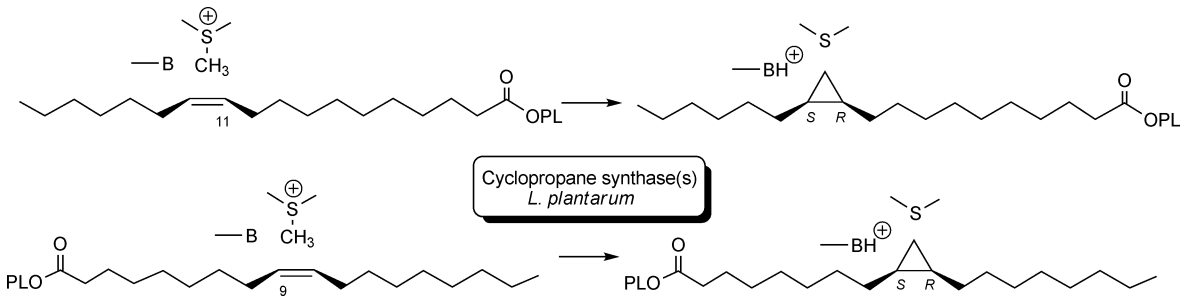
Scheme 4 Probing the stereochemistry of cyclopropane ring formation using chiral methyl-S.A.M.

chiral methyl acetic acid led to the conclusion that the base responsible for proton abstraction was located on the methyl terminus side of the incipient cyclopropane ring (Scheme 4).[69] This mechanistic picture is based on the reasonable assumption that the initial transfer of the methyl group from S.A.M. occurs with inversion (S_N2)[70] and subsequent deprotonation with retention. The latter has precedent in cyclopropane ring formation during cycloartenol biosynthesis.[71] Obligatory formation of a sulfur ylid during cyclopropanation would probably have resulted in complete loss of stereochemical information in a chiral methyl group experiment.

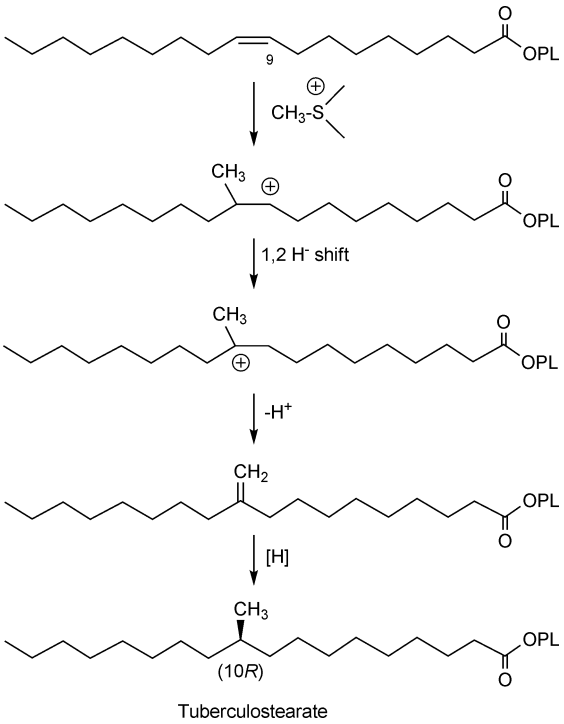
Further evidence for tight stereochemical control of reactive intermediates in the biosynthesis of cyclopropyl fatty acids was revealed in an unexpected fashion. Earlier studies had shown that when a series of regioisomeric (*Z*)-octadecenoates were incubated with an *E. coli* fatty acid auxotroph, only the 9,10 (oleate) and 11,12 (*cis*-vaccenate) regioisomers were methylenated (Scheme 5).[72] A similar phenomenon was noted in the case of *L. plantarum* (P. H. Buist, unpublished results). An attempt was made to pinpoint the location of the carbocation formed during cyclopropanation of each regioisomer using pairs of homoallylically monofluorinated oleates and homoallylically monofluorinated *cis*-vaccenates.[73] The pattern of fluorine-induced rate retardations that was obtained led to the prediction and subsequent confirmation that the two parent regioisomeric cyclopropyl products shown were in fact quasienantiomeric as shown in Scheme 5.[74] The methodology for determining the absolute configuration of these cyclopropyl fatty acids had previously been worked out by Tocanne and requires chiroptical evaluation of the corresponding α-ketocyclopropyl compounds.[75] The results of the Arigoni chiral methyl test when applied to oleate cyclopropanation in *L. plantarum* confirmed that the enzymic base is located at the C-1 end of a 9,10-olefin (Scheme 5).[74] In an effort to establish the generality of this bimodal regioselectivity, the stereochemistry of cyclopropyl fatty acids obtained from *E. coli* and other sources is being studied.[76]

2.1.2 Tuberculostearic acid biosynthesis. First isolated in 1929 by Anderson and Chargaff,[77] tuberculostearic acid ((*R*)-10-methyloctadecanoic acid, T.S.A.) is a component of the complex cell wall that contributes to the survival of *Mycobacterium tuberculosis* within its human host and serves as a useful diagnostic marker for tuberculosis.[78] The (*R*)-configuration was assigned to C-10 of T.S.A. on the basis of chiroptical data with the help of synthetic reference standards.[79] Two syntheses of highly enantiomerically enriched T.S.A. have been reported recently and confirm the orginal stereochemical assignment.[80,81] The biological role of methyl-substituted fatty acids such as T.S.A. is not clear; *a priori* one would speculate that methylation would tend to disorder cell membranes by disturbing interchain packing through steric interference.

The mechanistic pathway leading to tuberculostearate formation is similar to that found for the formation of 24-methyl sterols[32] in that methyl transfer to olefin is followed by a 1,2-hydride shift and subsequent deprotonation (Scheme 6). The exomethylene intermediate is then reduced. Early work by Law and coworkers[82] on the enzymology of 10-methylenation revealed striking overall similarities with cyclopropane synthase (*vide supra*) and further substantiated the view that a common carbocationic intermediate might be involved as outlined in Scheme 2 (pathways a and c). The absence of a properly-positioned basic group required to form a cyclopropane ring from the initially formed carbocationic intermediate would allow a 1,2 H^- shift to occur followed by proton abstraction from below the original olefinic plane. The validity of such a proposal has been supported by the results of experiments using chiral methyl group S.A.M. (Scheme 7) generated *in vivo* by *Mycobacterium phlei*.[83] Synthesis and *in vivo*



Scheme 5 Biosynthesis of quasienantiomeric cyclopropane fatty acyl regioisomers in *Lactobacillus plantarum*.

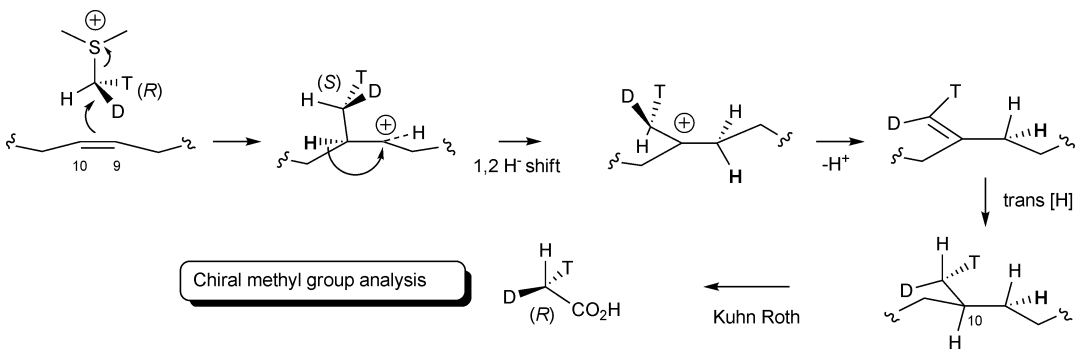


Scheme 6 Tuberculostearate formation in *Mycobacteria* proceeds *via* an exomethylene intermediate.

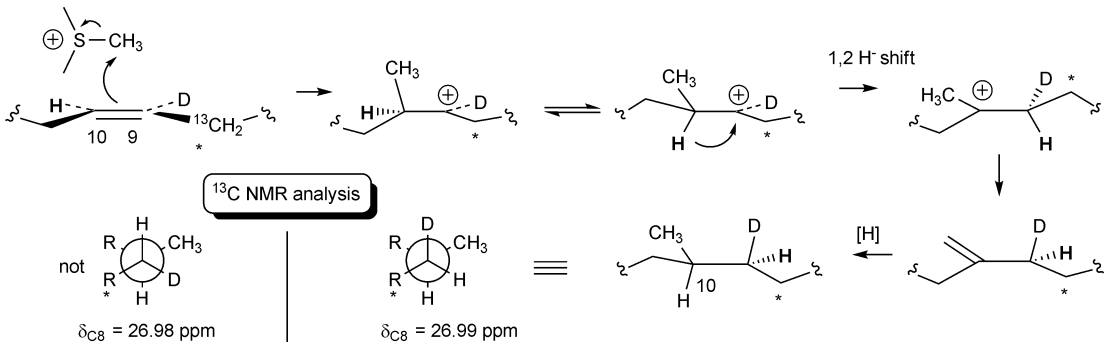
bioreduction of the multiply-labelled exomethylene intermediate was required to deduce the stereochemistry of proton removal from the C-10 carbocationic intermediate. The stereochemical details of the 1,2-hydride shift were established in elegant fashion by incubating a 9-deuterooleate substrate with *M. phlei* and determining the absolute configuration of the C_9DH group (*S*) of the product (Scheme 8). The latter could be accomplished by taking advantage of the fact that the β-isotope shift on the ^{13}C resonance of a C-8 reporter atom is slightly different for diastereomeric C-9-d_1 isotopomers. The stereochemical details for tuberculostearate biosynthesis are similar to that found for sterol 24-methyltransferase.[84]

2.1.3 Sphingolipid C-methylation. C-methylation of an (*E*)-olefinic fatty acid derivative has been confirmed in glucosylceramide biosynthesis as it occurs in the yeast, *Pichia pastoris* (Scheme 9).[85] Compounds of this type are thought to play an important role in plant–pathogen interactions, interestingly, plant or animal glucosylceramide do not bear the unique C-9-methyl signature. Sequence analysis of the *Pichia* methyltransferase showed that this protein belongs to the superfamily of *S*-adenosylmethionine-(S.A.M.)-dependent methylases, a high sequence similarity to plant and bacterial cyclopropane fatty acid synthases was also found. A minimal mechanism for C-methylation in the *Pichia* system is given in Scheme 9: proton loss from C-9 of the putative carbocationic intermediate occurs presumably from below the plane of the original alkene to give an internal olefin.

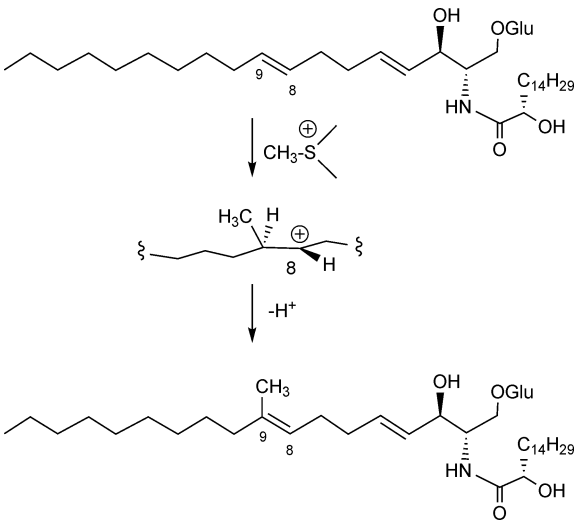
2.1.4 Mycolic acid biosynthesis. As alluded to in the Introduction, the detailed study of mycolic acid (Fig. 1) biosynthesis in *Mycobacteria* is being pursued with renewed vigour due to the urgent need to find new targets for antitubercular drug development. A most welcome development in this line of research has been the publication of enzyme crystal structures relating to



Scheme 7 Probing the stereochemistry of tuberculostearate formation using chiral methyl-S.A.M.



Scheme 8 Use of stereodependent ^{13}C NMR deuterium isotope effects to track stereochemistry of 1,2-hydride shift in tuberculostearate biosynthesis.

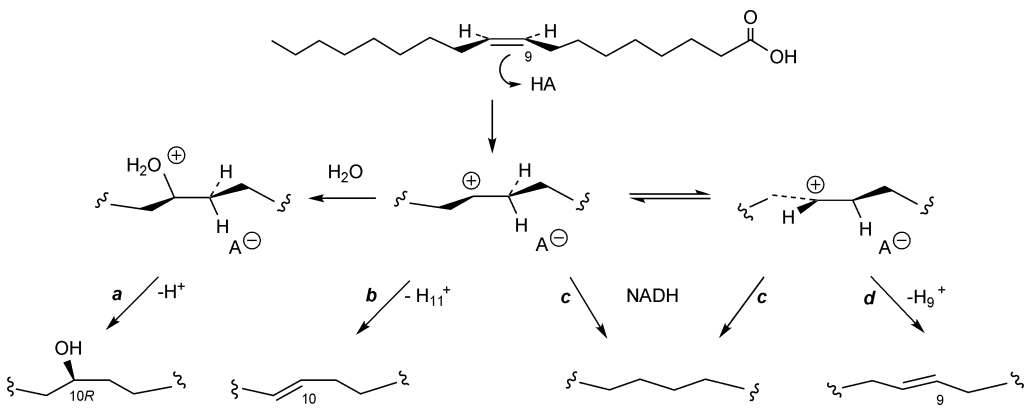


Scheme 9 C-methylation of unsaturated sphingolipids by a *Pichia pastoris* methyltransferase expressed in *Saccharomyces cerevisiae*.

cyclopropane ring formation (cma1, cma2 and PcaA).[57] However, a major stumbling block to further progress in this area is the difficulty of obtaining *in vitro* activity for these systems. The new structural data has allowed homology modeling of other cyclopropane synthases and related methyltransferases for the first time and new insights regarding modes of substrate binding can be expected to emerge from this research.[85] A particularly interesting subgroup of the mycolic acid family of compounds contains the CH_2–$CH(CH_3)$–$CH(OCH_3)$–CH_2 moiety, this methylation pattern is thought to arise by carbocation quenching with water (Scheme 2, pathway e) followed by *O*-methylation of the hydroxyl intermediate. The enzyme responsible for this unique transformation (HmA (MmaA4)) has recently been characterized by X-ray crystallography.[86] Notably, the bicarbonate ion found in structurally related cyclopropane synthases is absent in the HmA structure and is replaced by a topologically equivalent, glutamate H-bonded water molecule.

2.2 Hydration

The regio- and stereoselective enzyme-catalyzed hydration of oleic acid is a remarkable reaction and inspired, in part, an early effort to imitate enzymic efficiency by designing and constructing a purely synthetic catalyst.[87] The formation of (*R*)-10-hydroxystearate (10-H.S.A., Scheme 10, pathway a) is a remarkably widespread phenomenon among bacteria and other microorganisms although its biological purpose is obscure. A related compound, (*S*)-8-OH palmitate is reported to be an endogenous inhibitor of spore germination in *Lygodium japonicum*.[88] A cautionary note has been issued with respect to detection of 10-hydroxystearate in lipid extracts, the occurrence of this compound may be traced to contamination by *Pseudomonas* species.[89]



Scheme 10 Hydration of oleic acid and relationship to other olefin additions possibly initiated by protonation.

The essential mechanistic details of oleate hydration in a *Pseudomonas* species have been worked out with the help of isotopic labeling experiments: *anti* addition of water (Scheme 10, pathway a), probably initiated by a protonation step, was observed.[90] A minor pathway involving a reversible double bond shift by abstraction of the C11 proton (Scheme 10, pathway b) was also identified. Attempts to further characterize this hydratase system have been unsuccessful.[91] It is interesting to note the similarity in regiochemical and stereochemical preference between oleate 10-hydratase and the well-characterized soybean epoxide hydrolase.[92] The latter enzyme uses a two step mechanism involving nucleophilic attack of an H-bonded 9,10-epoxide by an aspartate residue followed by hydrolysis of the covalently bound intermediate. Two tyrosine residues are thought to provide general acid catalysis.

Given the commercial importance of chiral hydroxyfatty acids, the biotechnological aspects of oleate hydration have been explored.[93] The corresponding lactone of 10-H.S.A. is an important flavour-impact compound.[94] *In vivo*, hydroxyfatty acids tend to be in equilibrium with the corresponding keto compounds *via* a rapid, reversible dehydrogenation process and this circumstance lowers the % ee of biosynthetic 10-H.S.A. A convenient method for evaluating the enantiomeric purity of 10-H.S.A. *via* the ^{1}H NMR of chiral mandelate derivatives has been reported.[95]

2.3 *Cis–trans* isomerization

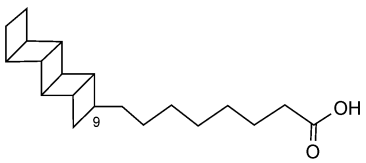
Conversion of (*Z*)-unsaturated fatty acids to the corresponding (*E*)-isomers is a strategy used by *Pseudomonas* and *Vibrio* species to lower the fluidity of cellular lipid structures under membrane-destabilizing culture conditions such as elevated temperatures or the presence of organic solvents.[96] The latter condition frequently arises in applications such as bioremediation and biocatalytic synthesis. Comparison of the sequences of seven known *cis–trans* isomerase ("Cti") proteins has identified a heme-binding domain similar to that found in cytochrome c.[97] Consequently, an anaerobic, iron-mediated redox mechanism for isomerization has been proposed. This scheme involves a reversible 1 electron oxidation of olefin to radical cation to permit subsequent bond rotation around the C-9,10 bond.[98,99] Alternatively, one could imagine a mechanism involving a reversible protonation process (Scheme 10, pathway d). In this scenario, the carbocation-bearing half of the molecule would have to rotate given that isomerization occurs without loss of deuterium from a 9,10-d_2-oleate substrate.[98] A third mechanistic possibility would involve reversible addition of an anaerobically-generated protein-bound radical.

2.4 Hydrogenation

The biohydrogenation of olefinic fatty acids takes place in ruminant organisms such as *Butyrivibrio* species and is formally the reverse of fatty acid desaturation. Essentially no detailed mechanistic work has been carried out on this system since early work by Tove and coworkers on the enzymology of (9*Z*,11*E*)-octadecadienoate reductase—a nonheme iron-containing enzyme that selectively reduces the 9,10-olefinic linkage.[100] Competing isomerization and hydration processes compromise the use of mixed ruminal cultures for mechanistic studies.[101,102] In principle, an *anti* proton addition–NADPH quench sequence (Scheme 10, pathway c) can be envisaged for biohydrogenation. This scheme is similar to that proposed for sterol double bond reduction[40,103,104] where it is known that an epoxide analogue can be reduced directly by NADPH.

3 Ladderanes

The discovery and structural elucidation of the ladderanes (Fig. 2) in ammonia-oxidizing bacteria by a team at the Royal Netherlands Institute for Sea Research ranks as one of the most exciting developments in membrane biochemistry in recent years.[105] These highly strained compounds are linked to glycerol *via* ether bonds[106] and form a dense lipid-based protective membrane for a specialized internal compartment wherein ammonia oxidation to nitrate takes place. In this manner, diffusion of toxic intermediates such as hydrazine is prevented. The linearly fused cyclobutyl units may be biosynthesized by cyclization of an unsaturated lipid precursor. The possible involvement of S.A.M.-radical enzymes[25] (*vide infra*) in ladderane construction has been inferred from a recent genomic study.[107] The solution of this intriguing biosynthetic problem will certainly constitute a unique chapter in the history of natural products research.



Pentacycloanammoxic acid (*Candidatus* sp.)

Fig. 2 A unique ladderane fatty acid isolated from an anammox microbe.

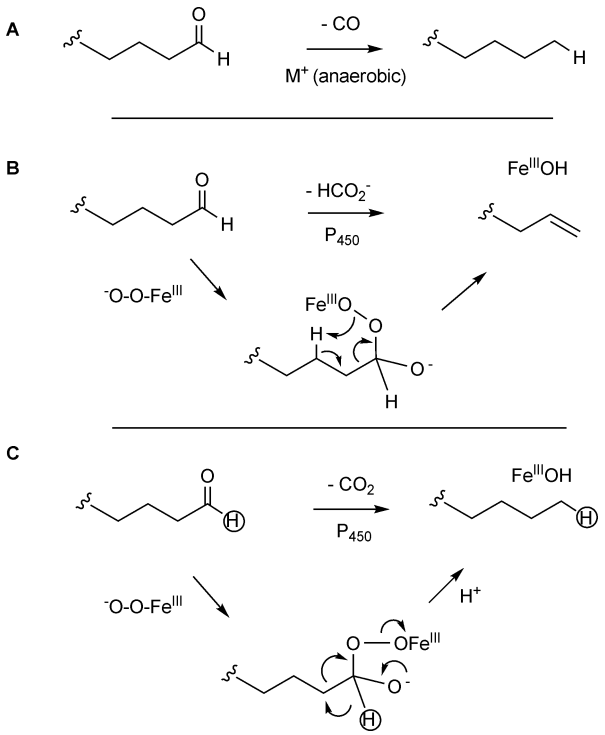
The absolute stereochemistry of the ladderane shown in Fig. 2 has not been determined due to lack of sufficient material but the tools for doing so have already been put in place.[108–110]

4 Hydrocarbon biosynthesis

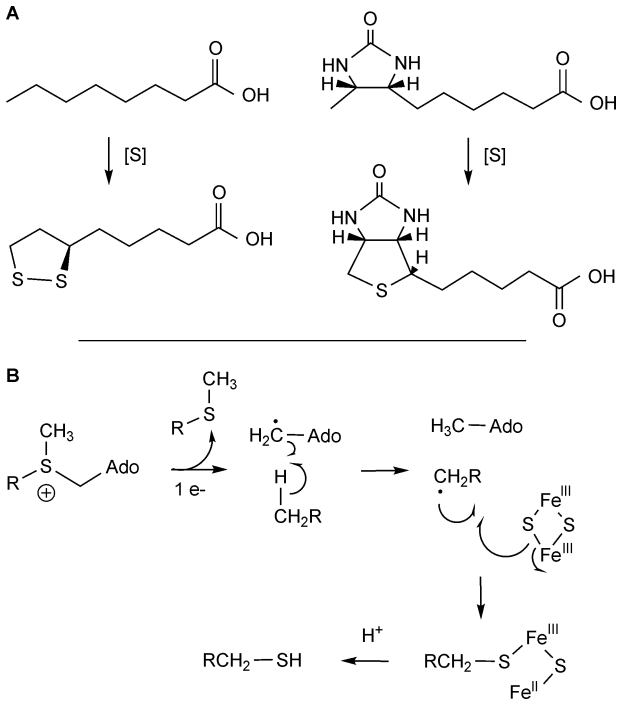
Formation of hydrocarbons from the corresponding fatty acids plays an important role in cuticular wax formation by plants,[111] insect pheromone biosynthesis[11] and the generation of allelochemicals such as falcarindiol (Fig. 3), (*vide infra*).[112] In addition, certain algae such as *Botryococcus braunii* accumulate large quantities of hydrocarbons that potentially constitute a renewable source of energy.[8,113] It is thought that loss of a terminal C1 unit may take place *via* an aldehyde—the product of carboxylic acid reduction. Three pathways have been postulated for α-carbonyl cleavage (Scheme 11A–C) in lipid biochemistry.

A. Anaerobic Co- or Cu-mediated direct extrusion of carbon monoxide.[114,115]

B. A nucleophilic P450 iron hydroperoxide adds to the aldehydic carbonyl and the adduct collapses by oxygen–oxygen scission to ultimately yield a terminal alkene and formate as shown in the "cartoon" mechanism (Scheme 11B). This oxidative mechanism has also been proposed for sterol demethylases including aromatase and 14-demethylase.[116,117]



Scheme 11 Hydrocarbon biosynthesis by **A** loss of carbon monoxide, **B** formate or **C** carbon dioxide.



Scheme 12 **A** The final thiolation step in lipoate and biotin biosynthesis. **B** S.A.M.-dependent mechanism of thiolation.

C. The adduct between the ferryl hydroperoxide and carbonyl terminus collapses *via* migration of the aldehydic hydrogen to the adjacent methylene group. This formal "mechanism" can be drawn for an insect P450-based decarbonylase based on the results of a deuterium labeling study.[118,119] This scheme is consistent with the observed retention of deuterium labels at C1, C2 and C3 of the original substrate and the release of carbon dioxide rather than formate or carbon monoxide.

Further information on these enigmatic processes should be forthcoming in the context of extensive efforts to identify the genes involved in cuticular wax biosynthesis[111]

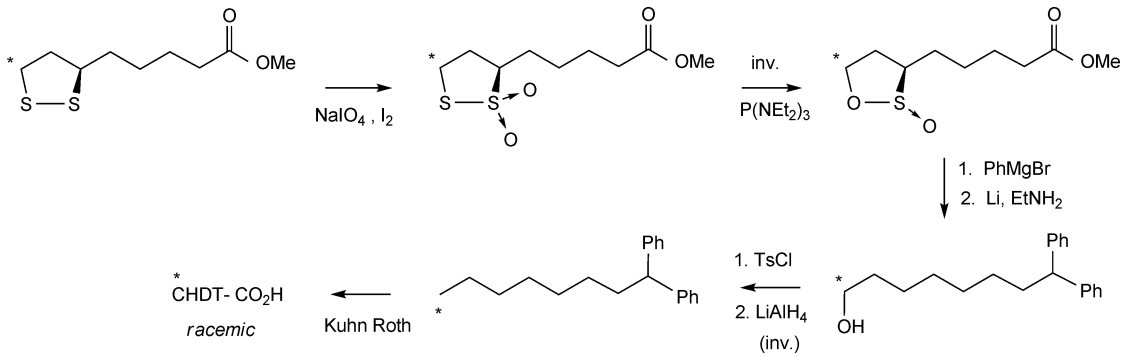
5 Thiolation

The final "sulfur insertion" step in the biosynthesis of lipoic acid and biotin (Scheme 12A) and perhaps other sulfur-containing compounds[120] remained a mystery for many years until the involvement of S.A.M. and iron sulfur clusters in these processes was established.[25–28,121,122] When the first stereochemical studies on sulfur insertion were being carried out by Parry and coworkers,[123] cleavage of unactivated C–H bonds were thought to be the sole domain of O_2-dependent metalloenzymes such as cytochrome P450. However, incorporation of possible hydroxy precursors gave poor results and thus a sequence involving hydroxylation–sulfide displacement of an activated alcohol leaving group did not appear to be involved in the biosynthesis of lipoic acid or biotin. In fact, the mode of sulfur insertion turned out to be far more elegant[124] (Scheme 12B): 1 electron reduction of S.A.M. by an enzyme-bound iron sulfur cluster is thought to generate the highly reactive adenosyl radical. This species is competent to abstract a hydrogen from the substrate and the resultant carbon-centred radical captures sulfur from a second iron sulfur cluster. A recent crystal structure of biotin synthase and sequence comparisons of lipoate synthase and biotin synthase confirmed that both enzymes belong to the same AdoMet radical family of proteins and share a strongly conserved secondary structure.[125,126] On this basis, one can now interpret results of stereochemical investigations that were initiated three decades ago.

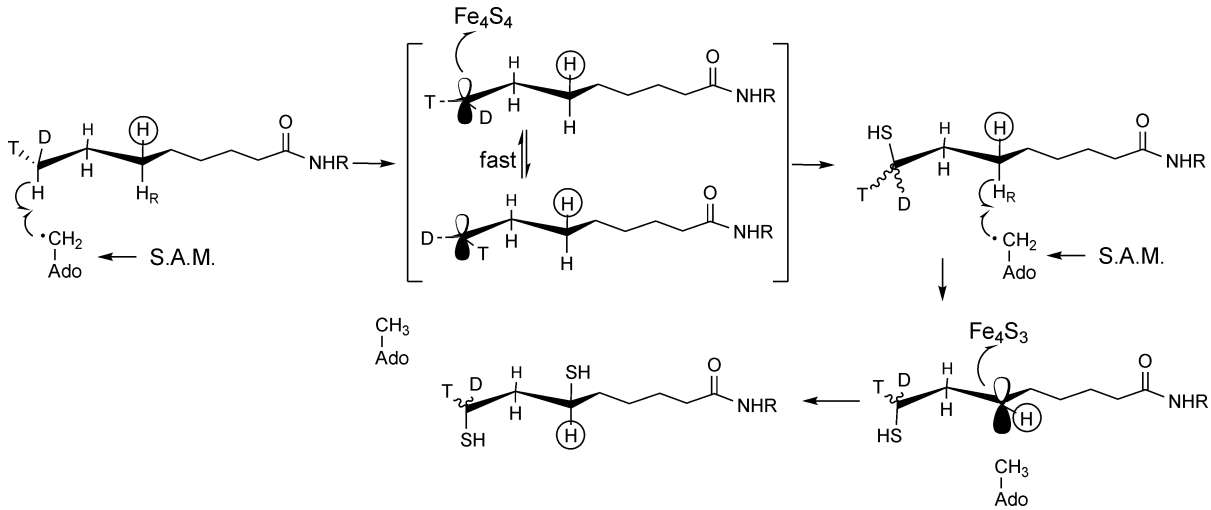
5.1 Lipoate synthase

Pioneering labeling experiments using an *in vivo E. coli* lipoate biosynthesizing system[123] established that thiolation at C-6 and C-8 of octanoic acid took place without involvement of unsaturated precursors. In addition, sulfur "insertion" at C-6 was shown to occur with inversion of configuration by analyzing the tritium content of products derived from stereospecifically C-6 tritiated octanoates. Thiolation at C-8 was shown to occur with racemization by incubating chiral (*R*)-methyl octanoic acid with *E. coli* and degrading the resultant biosynthetic lipoate in a stereocontrolled manner as shown in Scheme 13.[127] The key step in the degradation sequence was the use of Harpp chemistry[128] to replace the C8-sulfur bond with a C8-oxygen bond—a process shown to occur with clean inversion in control experiments. Employing this strategy allowed one to use the well-characterized tosylation–$LiAlH_4$ reduction sequence in order to generate a methyl-group bearing degradation product suitable for chiral methyl group analysis.

The stereochemical results obtained for the thiolation steps in lipoate biosynthesis were puzzling at the time since oxygen insertion biochemistry was known to proceed primarily with



Scheme 13 Stereocontrolled degradation scheme of C-8-labelled lipoate biosynthesized from chiral methyl-labeled octanoate.



Scheme 14 Stereochemical aspects of sulfur "insertion" at C-6 and C-8 in lipoate biosynthesis.

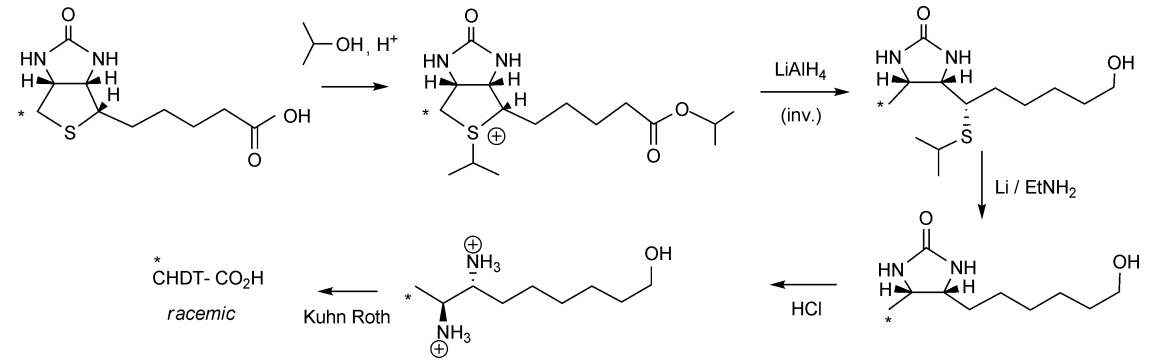
retention of configuration.[129,130] However, given what is now known about the nature of catalytic groups in lipoate synthase[131–137] and the closely related biotin synthase,[125,126] one can construct the following mechanistic scheme for bisthiolation by the former enzyme (Scheme 14). A C-8 methylene radical is generated by hydrogen abstraction from below the plane of the extended substrate, this intermediate undergoes rapid C-7,8 bond rotation before being quenched by an iron sulfur cluster residing above the plane of the radical. Booker and Cicchillo have shown that two equivalents of S.A.M. are required per lipoate produced[135] and thus a second S.A.M.-derived adenosyl radical abstracts the pro*R* hydrogen at C-6; the resultant secondary radical is quenched from above by the iron sulfur cluster to generate the thiol group with overall inversion of configuration.

5.2 Biotin synthase

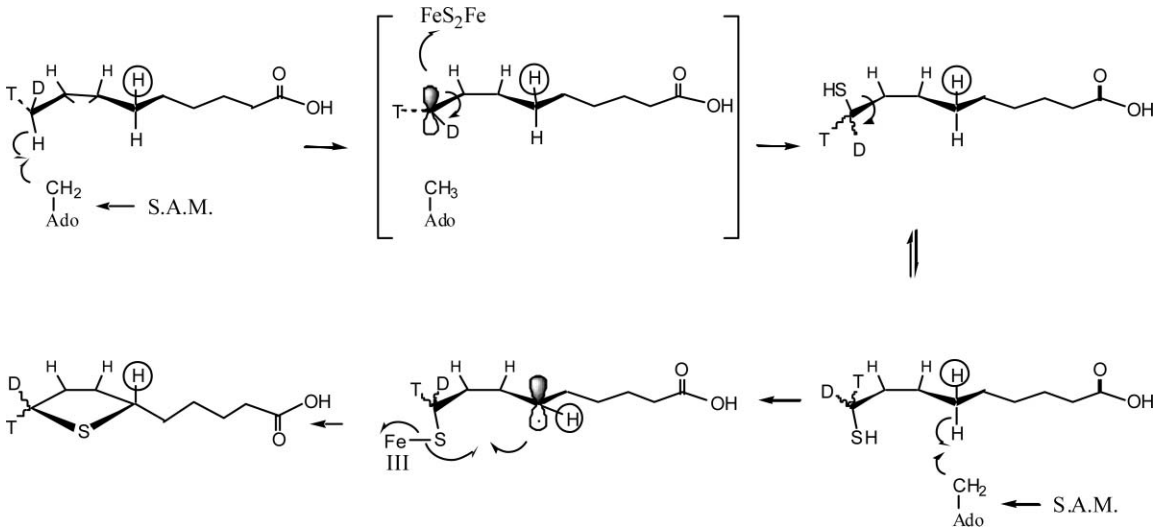
In vivo incubation experiments using *Aspergillus niger* as a biotin-synthesizing organism demonstrated that dehydrogenation–H_2S addition was not involved in the conversion of desthiobiotin to biotin.[123] These results pointed to a direct sulfur "insertion" mechanism similar to that observed in lipoate biosynthesis (*vide supra*). However unlike the lipoate case, sulfur introduction at C-6 was shown to occur with retention rather than inversion of configuration.[123] The stereochemistry of C-9 thiolation was studied using chiral methyl group methodology.[138] This undertaking involved synthesis of chiral methyl-desthiobiotin, incubation of this substrate with *A. niger*, and degradation of the multiply labeled biosynthetic product (Scheme 15) to a sample of acetic acid that was determined to be racemic at the methyl group, a result identical to that obtained subsequently for lipoate biosynthesis (*vide supra*). These results, as well those of the Marquet group and others[139–142] can be interpreted using the crystal structure of biotin synthase as a guide.[125] The Drennan structure locates the biotinyl substrate between two iron sulfur clusters, one of which is poised to reduce a proximal S.A.M. molecule to generate the adenosyl radical—the putative hydrogen abstracting species. A possible sequence of events for the desthiobiotin–biotin conversion is shown in Scheme 16 and would include abstraction of the C-9 hydrogen from below, thiolation of a rapidly rotating, methylene radical from above, abstraction of the C-6 hydrogen from below and capture of the C-9 sulfido group with overall retention of configuration at C-6. Further details on sulfur transfer will no doubt emerge from on-going mechanistic work on this remarkable enzyme.[143,144]

6 O_2-dependent C–H activation

Fatty acid hydroxylation and its mechanistic variant—desaturation (dehydrogenation) is an O_2 and NAD(P)H-dependent process (eqn (1),(2)):



Scheme 15 Stereocontrolled degradation scheme of C-9-labelled biotin biosynthesized from chiral methyl-labeled desthiobiotin.



Scheme 16 Stereochemical aspects of sulfur "insertion" at C-6 and C-9 in biotin biosynthesis.

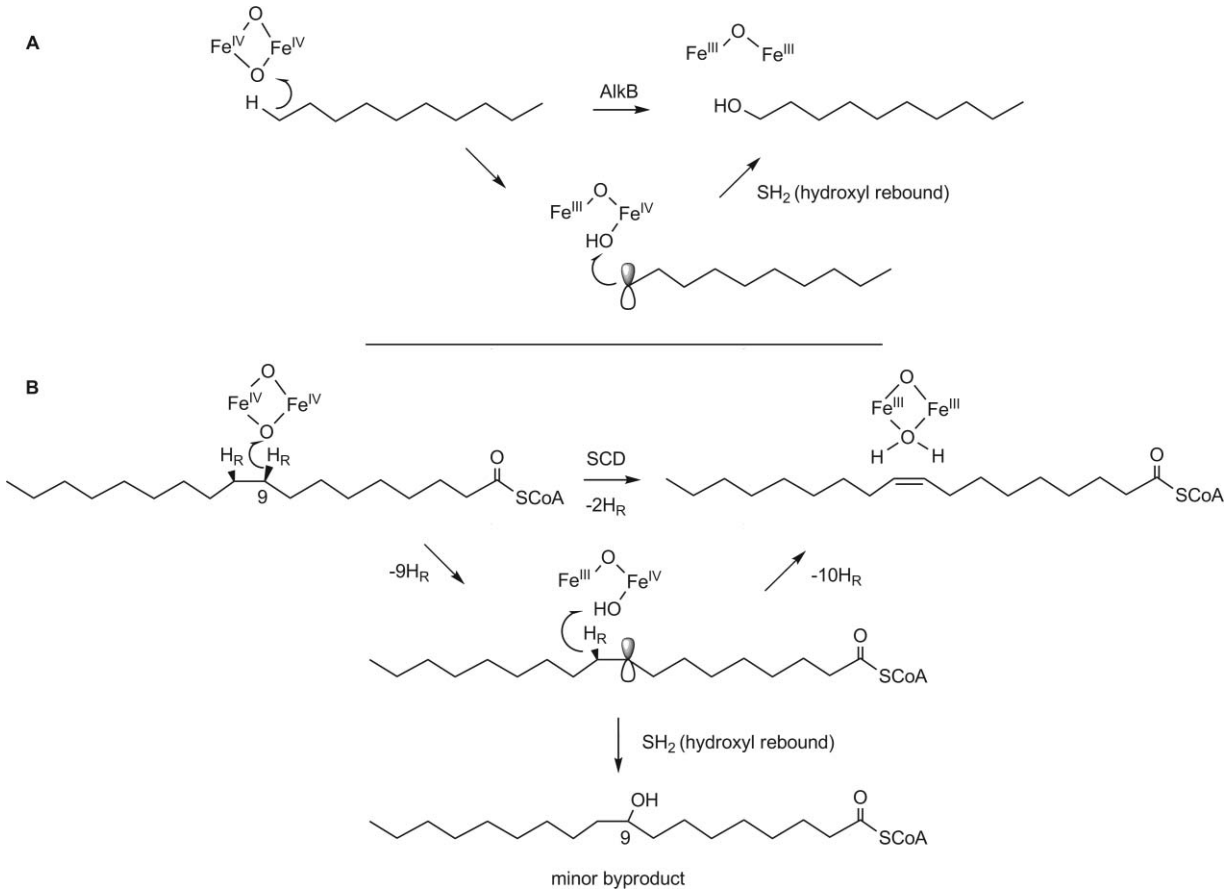
$$H^+ + NAD(P)H + O_2 + RCH_2{-}CH_2R' \rightarrow NAD(P)^+ + H_2O + RCH(OH){-}CH_2R' \quad (1)$$

$$H^+ + NAD(P)H + O_2 + RCH_2{-}CH_2R' \rightarrow NAD(P)^+ + 2\,H_2O + R{-}CH{=}CHR' \quad (2)$$

The enzymes catalyzing these reactions can be classified according to the nature of the prosthetic group that generates the active oxidizing species.[145] These are: 1) heme-coordinated iron found in cytochrome P450; 2) a carboxylate-bridged, MMO-type, non-heme diiron cluster characteristic of soluble desaturases, 3) a multi-histidine-coordinated non-heme diiron cluster residing in membrane-bound enzymes. Mechanistic work in this area has benefited greatly from recent experimental and theoretical scrutiny of prototypical systems such as cytochrome $P450_{cam}$ and methane monoxygenase.[145–148]

6.1 ω-Hydroxylase

Alkanes of medium chain length are hydroxylated at the terminal methyl group by alkane ω-monooxygenase (alkB, Scheme 17A). This reaction was first discovered in a hydrocarbon-metabolizing bacterium, *Pseudomonas oleovorans*, and has gained iconic status in the area of bioremediation and biochemical engineering. AlkB is also important because it remains the only enzyme of the large, membranous, non-heme diiron class to be characterized spectroscopically.[149,150] This analysis revealed the presence of a diiron centre in a histidine-rich co-ordination environment. Hydropathy analysis suggests that alkB is anchored by several membrane-spanning, α-helical segments and is similar in overall structure to the family of membrane-bound desaturases.[151] The results of site-directed mutagenesis experiments are beginning to define the topography of the active site required to permit selective attack of the methyl terminus.[152] Mechanistic studies using cyclopropyl radical clock methodology have revealed that short-lived radical intermediates are probably involved in the two step hydroxylation event (Scheme 17A).[153,154] The putative carbon-centred radical is captured very efficiently because hydroxylation occurs with retention of configuration as determined through the use of a chiral methyl substrate.[155] The recent characterization of CYP1533A6-soluble cytochrome P450 alkane ω-hydroxylator[156] will facilitate direct mechanistic comparison between a heme iron- and non-heme iron-containing enzyme with identical regioselectivity.



Scheme 17 Postulated mechanism for **A** ω-hydroxylation and **B** membranous Δ^9 desaturase-mediated dehydrogenation–9-hydroxylation.

6.2 Cytochrome P450 BM-3

Most cytochrome P450 research has been concerned with predicting and understanding how membrane-bound hepatic isozymes metabolize lipophilic xenobiotics including linear hydrocarbons.[157] Until recently, this work relied on the use of structural data obtained for soluble P450's. The publication of the first crystal structures of a membranous hepatic cytochrome P450[158,159] constitutes a major breakthrough in our understanding of P450 structure–function relationships and the scope and limitations of homology modeling.

Another major focus of current cytochrome P450 research is the potential use of these catalysts in synthetically useful oxygenation reactions on simple hydrocarbon substrates. To date, the protein of choice for such investigations has been bacterial cytochrome P450 BM-3, a soluble protein that hydroxylates fatty acids near the methyl terminus in an enantioselective manner. The regioselectivity profile of this enzyme is complementary to that of alkB and cytochrome P450 153A6 (*vide supra*) in that methylene groups proximal to the methyl terminus are oxidized rather than the methyl group. BM-3 is unique in that it does not require ancillary proteins to transfer reducing equivalents to the heme iron catalytic center. These characteristics make it ideal for consideration as a biocatalyst in synthesis applications and this protein has been targeted as a candidate for protein engineering.[160] Complementary to these studies is a substantial body of fundamental work investigating the detailed mechanism of BM-3 in the context of changing paradigms.[161–164]

6.3 Fatty acid desaturases

The bioorganic chemistry of fatty acid desaturases has been reviewed recently.[21] Most of the structural and detailed bioinorganic work in this area has been carried out using the soluble, non heme diiron MMO-type desaturase isolated from castor.[165] In addition, the mechanistic probes devised for the study of various membranous desaturases[166] have been applied with some success to the castor enzyme.[21] A major development currently driving research on desaturase function is the discovery that overexpression of mammalian stearoyl CoA Δ^9 desaturase (SCD) is associated with lifestyle disorders relating to the metabolic syndrome (obesity and type II diabetes).[167] The observation that a closely related yeast SCD homolog initiates Δ^9 desaturation by a kinetically important C-9 H abstraction step (Scheme 17B) may be useful in the development of selective inhibitors.[168] A second application is related to the discovery that a membranous Δ^9 desaturase (DesA3) in *Mycobacteria* may be a useful lipid-based target for anti-tubercular drugs.[169] An associated reductase has been characterized[170] and a soluble mycobacterial desaturase (DesA2) has also been characterized by X-ray crystallography.[171]

The critical mechanistic problem that remains to be understood more fully is the switch controlling desaturation and hydroxylation pathways (Scheme 17B). This issue has been addressed computationally for cytochrome P450—an enzyme that is normally a pure hydroxylator but can be tuned to act as a desaturase.[172] The latter pathway is apparently promoted by formation of a carbocationic intermediate that is sterically hindered to hydroxide rebound.[173] The relevance of this work to non-heme diiron systems remains to be established. For the latter enzymes, protein engineering experiments have shown that Δ^{12} desaturases can act as an enantioselective 12-hydroxylase when relatively conservative changes to amino acids proximal to the putative substrate binding site are made.[174] The seminal discovery of the Shanklin group that hydroxyfatty acids are formed as minor byproducts of many membrane-bound desaturases[174] has been extended to include an insect Δ^{11} desaturase.[175] Without exception, the regiochemistry of desaturase-mediated hydroxylation corresponds to the site of initial hydrogen removal in the corresponding desaturation pathway (Scheme 17B).[21] This observation is consistent with the intermediacy of a radical intermediate that can be steered towards hydroxyl rebound or a second H abstraction by stereoelectronic factors. Nowhere is the chemoselectivity of these systems more spectacularly displayed than in the case of alkyne formation (Scheme 1C, reaction 4) which features stepwise removal of adjacent hydrogens[176,177] and where epoxide formation (Scheme 1A, reaction 4) is prevented. Interestingly, the 12-acetylenase also functions as a normal desaturase using oleate (9*Z*-octadecenoate) as substrate but produces a mixture of (*E*/*Z*)-12,13-olefinic isomers.[178] Further insight into this intriguing mechanistic problem must await structural characterization of these and other membrane-bound desaturases.

6.4 Polyacetylene biogenesis

Polyacetylenic fatty acids or their derivatives constitute an important group of natural products with a wide spectrum of bioactivity.[179] Some prominent examples of this class of compounds are shown in Fig. 3. These include falcarindiol,[180] panaxytriol,[181] *Echinacea* diynes,[182] wyerone,[183] and obtusallene I.[184] A biogenetic scheme for falcarindiol biosynthesis has been proposed recently (Scheme 18);[112] modest variations in oxidative biochemistry would generate panaxytriol or a lauredial precursor *en route* to the interesting bromoallenic compound—obtusallene I (Scheme 18B).[185] All of the proposed enzymatic steps have precedent—alkyne[186] and terminal alkene formation,[187] hydroxylation[164] and reductive decarboxylation.[113] Identification of the various enzymes involved in these pathways represents a new frontier in phytochemistry.

7 Oxylipin biosynthesis

The lipoxygenase-mediated hydroperoxidation of polyunsaturated fatty acids initiates a cascade of interesting reactions leading to eicosanoids such as prostaglandins and leukotrienes in mammalian systems[188] and oxylipins in non-mammalian organisms.[189] Substantial progress has been made in accounting for the regio- and stereoselectivity of lipoxygenases using the tools of site-directed mutagenesis along with new structural information.[190–192] Some of these findings may prove to be useful as models for fatty acid binding to desaturases and methyltransferases (*vide supra*). For many years, the nature of the initial, rate-determining H-abstraction step in lipoxygenation was a matter of debate until inorganic modeling studies revealed that an unusual Fe^{III}-OH mediated PCET (proton coupled electron transfer) process was operating (Scheme 19).[193–195] The observation of an extremely high primary deuterium kinetic isotope effect for the initial C–H bond cleavage step is consistent with a tunneling process.[196] The kinetics of oxygen trapping of the putative radical intermediate have also been investigated.[197]

Further oxidative elaboration of hydroperoxide intermediates leads to a diverse array set of bioactive compounds with signaling and defensive properties.[198,199] In plants, a family of cytochrome P450 enzymes catalyzes a number of particularly interesting transformations including divinylether and allene oxide formation (Scheme 20AB). The allene oxide intermediate is processed to ultimately give jasmonic acid, an important signaling agent in the phytochemical stress response.[200] The mechanisms involved in divinylether and allene oxide formation are essentially variations on the elimination mechanism shown in Scheme 11B. All of these pathways are driven by what is thought to be a heme iron-induced fragmentation of the hydroperoxide substrate followed by proton loss from an intermediate carbocation.[201,202] Similar "electronic gymnastics" have been postulated by Ullrich in his consideration of the pathways involved in P450-catalyzed formation of prostacylins and thromboxanes.[203] An elegant stereochemical study showing that divinyl ether stereoisomer formation proceeds by

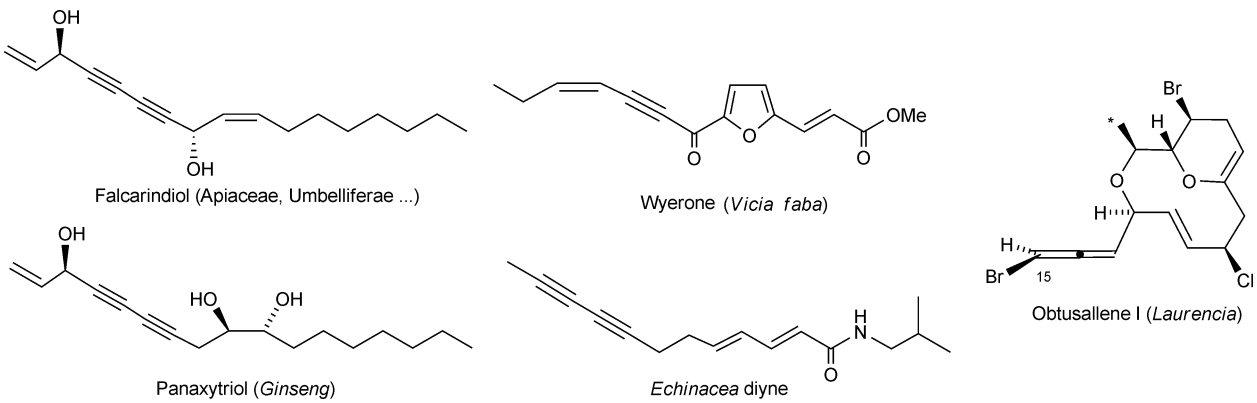
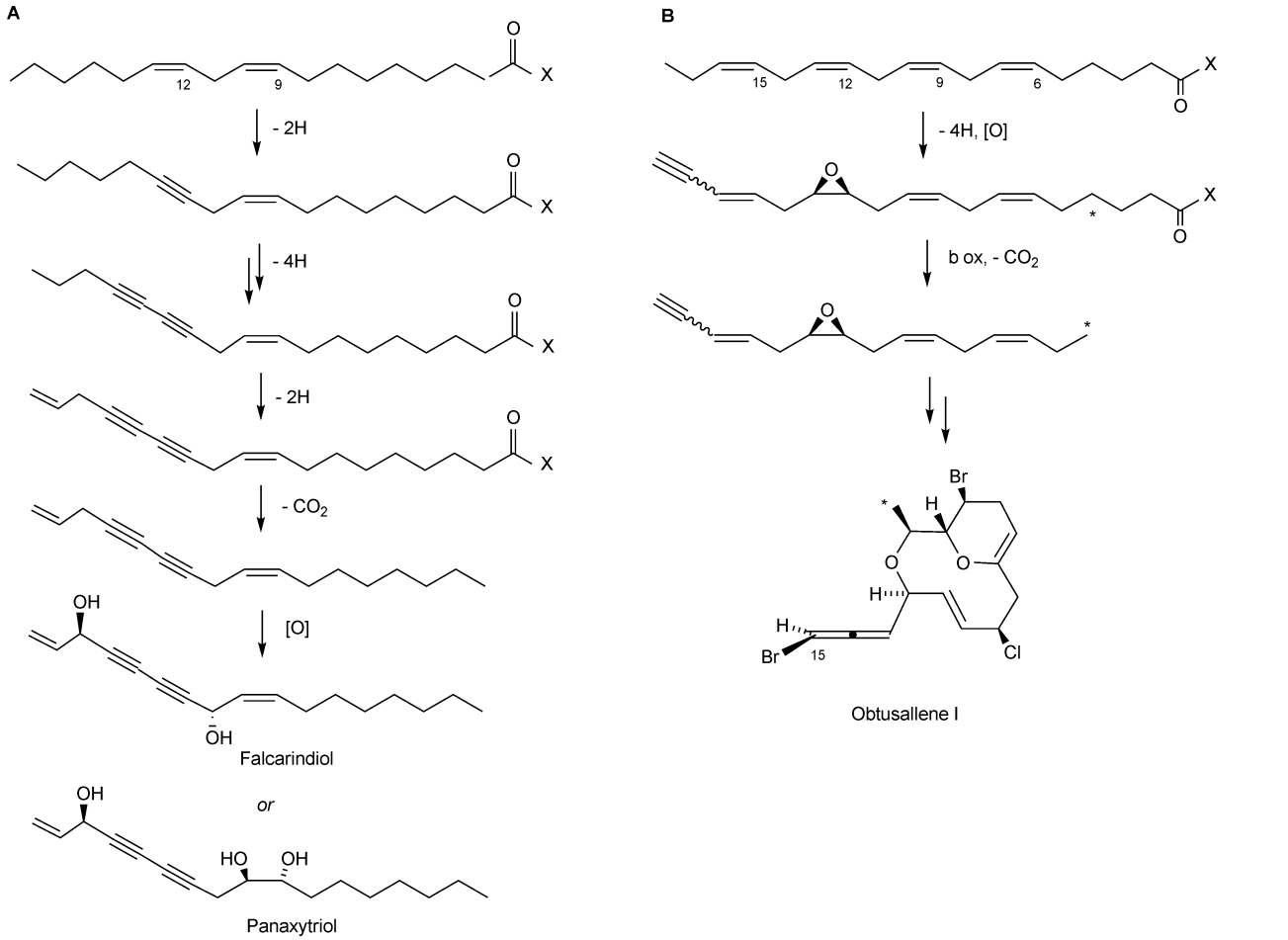
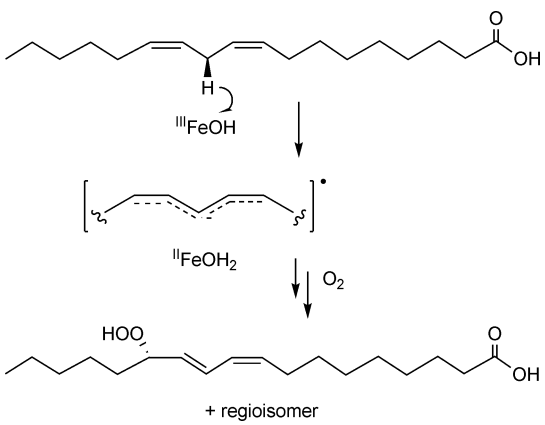


Fig. 3 Some exotic naturally occurring acetylenic fatty acids.



Scheme 18 **A** Biogenesis of bioactive diynoic hydrocarbons derived from linoleate. **B** Biogenesis of a cyclic halogenated natural product derived from a polyunsaturated fatty acid precursor.



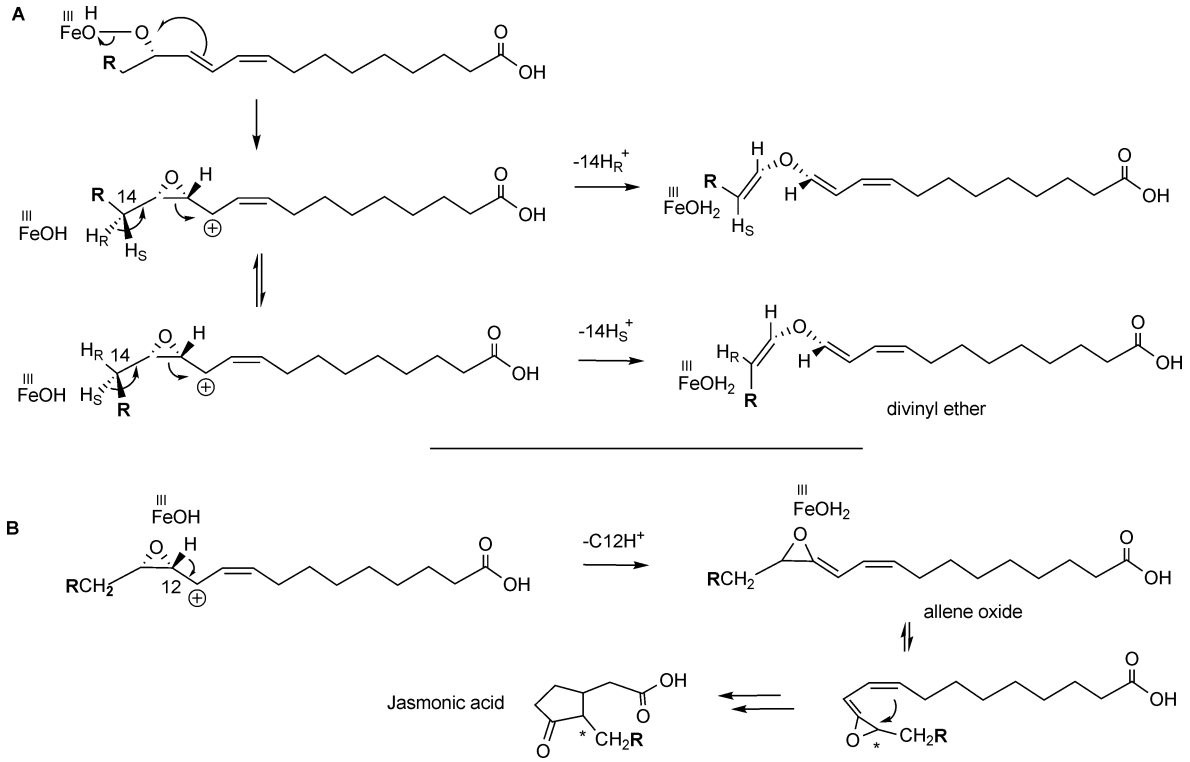
Scheme 19 Initial hydrogen abstraction step catalyzed by lipoxygenases.

loss of topological equivalent prochiral hydrogens (Scheme 20A) has been reported recently.[204]

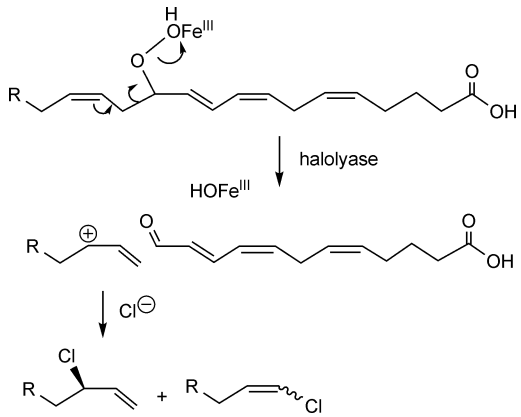
Given the intense current interest in halogenated natural products,[205–209] the recent discovery of a "lipoxygenase–hydroperoxide halolyase" pathway by Wichard and Pohnert (MPI Chemical Ecology, Jena) in the marine diatom, *Stephanopyrix turris* is worthy of special mention.[210] A possible mechanism for this transformation involves stereospecific quenching of the putative carbocationic intermediate by chloride ion (Scheme 21).

8 Summary

Spectacular advances in the structural biology of methyltransferases, desaturases, hydroxylases and thiolating enzymes have finally allowed interpretation of mechanistic results obtained previously using an *in vivo* approach. These results can now be used by researchers involved in modeling C–H activation and other synthetically important transformations. However, the details of other enzymatic reactions such as *cis–trans* isomerization, hydrogenation and decarbonylation remain obscure. Elucidating the biosynthesis of highly strained lipids such as cyclopropenyl and concatenated cyclobutylfatty acids (ladderanes) represent exciting new challenges to bioorganic chemists. We can also look forward to further progress in the structural biology of membrane-bound, lipid-modifying enzymes and the chemical biology of bioactive fatty acid derivatives such as polyacetylenes and oxylipins. *Post tenebras lux*.



Scheme 20 **A** Divinylether biosynthesis. **B** Allene oxide biosynthesis and cyclization.



Scheme 21 A possible mechanistic scheme for the halolyase pathway.

9 Acknowledgements

This review is dedicated, with thanks, to Duilio Arigoni and the "Arigonauten" of 1980–82.

10 References

1 C. E. Barry, III, R. E. Lee, K. Mdluli, A. E. Sampson, B. G. Schroeder and R. A. Slayden, *Prog. Lipid Res.*, 1998, **37**, 143.
2 A. Dobrzyn and J. M. Ntambi, *Prostaglandins, Leukotrienes Essent. Fatty Acids*, 2005, **73**, 35.
3 T. R. Dhiman, S. H. Nam and A. L. Ure, *Crit. Rev. Food Sci. Nutr.*, 2005, **45**, 463.
4 T. A. Taha, T. D. Mullen and L. M. Obeid, *Biochim. Biophys. Acta*, 2006, **1758**, 2027.
5 C. Somerville and J. Browse, *Science*, 1991, **252**, 80.
6 J. Jaworski and E. B. Cahoon, *Curr. Opin. Plant Biol.*, 2003, **6**, 178.
7 C. R. Somerville and D. Bonetta, *Plant Physiol.*, 2001, **125**, 168.
8 P. Metzger and C. Largeau, *Appl. Microbiol. Biotechnol.*, 2005, **66**, 486.
9 P. Broun, J. Shanklin, E. Whittle and C. Somerville, *Science*, 1998, **282**, 1315.
10 R. Jeffcoat and M. R. Pollard, *Lipids*, 1977, **12**, 480.
11 R. W. Howard and G. J. Blomquist, *Annu. Rev. Entomol.*, 2005, **50**, 371.
12 G. Pohnert and W. Boland, *Nat. Prod. Rep.*, 2002, **19**, 108.
13 M. J. Vasconcelles, Y. Jiang, K. McDaid, L. Gilooly, S. Wretzel, D. L. Porter, C. E. Martin and M. A. Goldberg, *J. Biol. Chem.*, 2001, **276**, 14374.
14 D. A. Los and N. Murata, *Biochim. Biophys. Acta*, 2004, **1666**, 142.
15 I. Inaba, B. Suzuki, Y. Szalontai, D. A. Kanesaki, H. Los, H. Hayashi and N. Murata, *J. Biol. Chem.*, 2003, **278**, 12191.
16 J. Browse and Z. Xin, *Curr. Opin. Plant Biol.*, 2001, **4**, 241.
17 D. W. Grogan and J. E. Cronan, *Microbiol. Mol. Biol. Rev.*, 1997, **61**, 429.
18 J. E. Cronan, *Curr. Opin. Microbiol.*, 2002, **5**, 202.
19 M. D. Kiran, S. Annapoorni, I. Suzuki, N. Murata and S. Shivaji, *Extremophiles*, 2005, **9**, 117.
20 J. S. Sinninghe Damste, M. Strous, W. I. Rijpstra, E. C. Hopmans, J. A. Geenevasen, A. C. van Duin, L. A. van Niftrik and M. S. Jetten, *Nature*, 2002, **419**, 708.
21 P. H. Buist, *Nat. Prod. Rep.*, 2004, **21**, 249.
22 B. J. Rawlings, *Nat. Prod. Rep.*, 1997, **14**, 335.
23 B. J. Rawlings, *Nat. Prod. Rep.*, 1998, **15**, 275.
24 Y. Sankawa, in *Comprehensive Natural Products Chemistry, Volume 1: Polyketides and Other Secondary Metabolites Including Fatty Acids and Their Derivatives*, ed. D. H. R. Barton, O. Meth-Cohn, K. Nakanishi and Y. Sankawa (volume editor), Elsevier, Oxford, 1999, pp. 1–22.
25 M. Fontecave, M. Atta and E. Mulliez, *Trends Biochem. Sci.*, 2004, **29**, 243.
26 M. Fontecave, *Nat. Chem. Biol.*, 2006, **2**, 171.
27 M. Fontecave, S. Ollagnier-de-Choudens and E. Mulliez, *Chem. Rev.*, 2003, **03**, 2149.

28 R. J. Parry, in *Comprehensive Natural Products Chemistry, Volume 1: Polyketides and Other Secondary Metabolites Including Fatty Acids and Their Derivatives*, ed. D. H. R. Barton, O. Meth-Cohn, K. Nakanishi and Y. Sankawa (volume editor), Elsevier, Oxford, 1999, pp. 825–864.
29 *Lipid Biochemistry: An Introduction*, ed. M. I. Gurr, J. L. Harwood and K. N. Frayn, Blackwell Science Ltd, Oxford, 5th edn, 2002.
30 *Biochemistry of Lipids, Lipoproteins and Membranes*, ed. D. E. Vance and J. E. Vance, Elsevier, Amsterdam, 4th edn, 2002.
31 *Lipid Metabolism in Plants*, ed. T. S. Moore, Jr., CRC Press, Boca Raton, 1993.
32 W. D. Nes, *Phytochemistry*, 2003, **64**, 75.
33 J. L. Giner, *Chem. Rev.*, 1993, **93**, 1735.
34 W. X. Zhou, Z. H. Song, J. L. Liu, M. B. Miller and W. D. Nes, *Tetrahedron Lett.*, 2004, **45**, 875.
35 A. Rahier, *Biochemistry*, 2001, **40**, 256.
36 B. S. Moore, in *Comprehensive Natural Products Chemistry, Volume 1: Polyketides and Other Secondary Metabolites Including Fatty Acids and Their Derivatives*, ed. D. H. R. Barton, O. Meth-Cohn, K. Nakanishi and Y. Sankawa (volume editor), Elsevier, Oxford, 1999, pp. 61–82.
37 E. Lederer, *Q. Rev. Chem. Soc.*, 1969, **23**, 453.
38 Y. Yuan and C. E. Barry, III, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 12828.
39 M. Julia, B. McDonald and M. C. Rezende, *Tetrahedron*, 1991, **47**, 6939.
40 M. Akhtar and C. Jones, *Tetrahedron*, 1978, **34**, 813.
41 D. A. Dougherty, *Science*, 1991, **271**, 163.
42 J. L. Giner, P. Buze and P. V. Schleyer, *J. Am. Chem. Soc.*, 1995, **117**, 12871.
43 P. Gutta and D. J. Tantillo, *J. Am. Chem. Soc.*, 2006, **128**, 6172.
44 S. P. T. Matsuda, W. K. Wilson and Q. B. Xiong, *Org. Biomol. Chem.*, 2006, **4**, 530.
45 K. Hofmann and R. A. Lucas, *J. Am. Chem. Soc.*, 1950, **72**, 4328.
46 V. R. Williams and E. A. Fieger, *J. Biol. Chem.*, 1946, **166**, 335.
47 W. W. Christie, in *Topics in Lipid Chemistry*, ed. F. D. Gunstone, Wiley, NY, 1970, ed. vol. 1, pp. 1–49.
48 M. S. Kuo, R. J. Zielinski, J. I. Cialdella, C. K. Marschkek, M. J. Dupuis, G. P. Li, D. A. Kloosterman, C. H. Spilman and V. P. Marshall, *J. Am. Chem. Soc.*, 1995, **117**, 10629.
49 X. M. Bao, S. Katz, M. Pollard and J. Ohlrogge, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 7172.
50 B. Perly, I. C. P. Smith and H. C. Jarrell, *Biochemistry*, 1985, **24**, 1055.
51 B. Jing, N. Tokutake, D. H. McCullough, 3rd and S. L. Regen, *J. Am. Chem. Soc.*, 2004, **126**, 15344.
52 H. T. Richard and J. W. Foster, *Adv. Appl. Microbiol.*, 2003, **52**, 167.
53 J. R. Warren, in *The Nobel Prizes 2005*, ed. K. Grandin, Nobel Foundation, Stockholm, 2006.
54 M. Haque, Y. Hirai, K. Yokota, N. Mori, I. Jahan, H. Ito, H. Hotta, I. Yano, Y. Kanemasa and K. Oguma, *J. Bacteriol.*, 1996, **178**, 2065.
55 V. Rao, N. Fujiwara, S. A. Porcelli and M. S. Glickman, *J. Exp. Med.*, 2005, **21**, 535.
56 M. S. Glickman, J. S. Cox and W. R. Jacobs, Jr., *Mol. Cell*, 2000, **5**, 717.
57 C. C. Huang, C. V. Smith, M. S. Glickman, W. R. Jacobs, Jr. and J. C. Sacchettini, *J. Biol. Chem.*, 2002, **277**, 11559.
58 F. Courtois and O. Ploux, *Biochemistry*, 2005, **18**, 13583.
59 D. F. Iwig, A. Uchida, J. A. Stromberg and S. J. Booker, *J. Am. Chem. Soc.*, 2005, **127**, 11612.
60 T. Cohen, G. Herman, T. M. Chapman and D. Kuhn, *J. Am. Chem. Soc.*, 1974, **96**, 5627.
61 D. F. Iwig, A. T. Grippe, T. A. McIntyre and S. J. Booker, *Biochemistry*, 2004, **43**, 13510.
62 F. Courtois, C. Guerard, X. Thomas and O. Ploux, *Eur. J. Biochem.*, 2004, **271**, 4769.
63 M. F. Hegazi, R. T. Borchardt and R. L. Schowen, *J. Am. Chem. Soc.*, 1979, **101**, 4359.
64 P. H. Buist and D. B. Maclean, *Can. J. Chem.*, 1982, **60**, 371.
65 T. Miwa, W. A. Garland, B. J. Hodshon, A. Y. H. Lu and D. B. Northrop, *J. Biol. Chem.*, 1980, **255**, 6049.
66 D. F. Iwig and S. J. Booker, *Biochemistry*, 2004, **43**, 13496.
67 E. J. Molitor, B. M. Paschal and H. W. Liu, *ChemBioChem*, 2003, **5**, 1352.
68 R. A. Pascal, S. J. Mannarelli and D. L. Ziering, *J. Biol. Chem.*, 1986, **261**, 2441.
69 J. P. Obrecht, Diss. ETH # 6983, 1982.
70 H. G. Floss and S. S. Lee, *Acc. Chem. Res.*, 1993, **26**, 116.
71 L. J. Altman, C. Y. Han, A. Bertolino, G. Handy, D. Laungani, W. Muller, S. Schwartz, D. Shanker, W. H. D. Wolf and F. Yang, *J. Am. Chem. Soc.*, 1978, **100**, 3235.
72 J. B. Ohlrogge, F. D. Gunstone, I. A. Ismail and W. E. Lands, *Biochim. Biophys. Acta*, 1976, **431**, 257.
73 P. H. Buist and R. A. Pon, *J. Org. Chem.*, 1990, **55**, 6240.
74 S. Rasyoni, Diss. ETH # 11318, 1995.
75 J. F. Tocanne, *Tetrahedron*, 1972, **28**, 363.
76 L. J. Stuart, J. P. Buck, A. E. Tremblay and P. H. Buist, *Org. Lett.*, 2006, **8**, 79.
77 J. Anderson and E. Chargaff, *J. Biol. Chem.*, 1929, **85**, 77.
78 G. L. Daikos, J. B. Brooks, A. Michos, E. Roma and V. Syriopoulou, *J. Tuberc. Lung Dis.*, 2004, **8**, 1027.
79 F. S. Prout, J. Cason and A. W. Ingersoll, *J. Am. Chem. Soc.*, 1948, **70**, 298.
80 O. Roberts and M. S. Baird, *Chem. Phys. Lipids*, 2006, **142**, 111.
81 X. Liu, B. L. Stocker and P. H. Seeberger, *J. Am. Chem. Soc.*, 2006, **128**, 3638.
82 Y. Akamatsu and J. H. Law, *J. Biol. Chem.*, 1970, **245**, 701.
83 P. Hanselman, Diss. ETH # 8334, 1987.
84 M. Mihailovic, Diss. ETH # 7535, 1985.
85 P. Ternes, P. Sperling, S. Albrecht, S. Franke, J. M. Cregg, D. Warnecke and E. Heinz, *J. Biol. Chem.*, 2006, **281**, 5582.
86 B. F. Boissier, F. Bardou, V. R. Guillet, S. Uttenweiler-Joseph, M. Daffe, A. Quemard and L. Mourey, *J. Biol. Chem.*, 2006, **281**, 4434.
87 J. W. Cornforth, *Proc. R. Soc. London*, 1978, **203**, 101.
88 Y. Masaoka, M. Sakakibara and K. Mori, *Agric. Biol. Chem.*, 1982, **46**, 2319.
89 P. Adam, K. Hannemann, J. Reiner and G. Spiteller, *Z. Naturforsch., C*, 2000, **55**, 965.
90 W. G. Niehaus, A. Kisic, A. Torkelson, D. J. Bednarczyk and G. J. Schroepfer, *J. Biol. Chem.*, 1970, **245**, 3790.
91 C. T. Hou, *J. Am. Oil Chem. Soc.*, 1995, **72**, 1265.
92 E. Blee, S. Summerer, M. Flenet, H. Rogniaux, A. Van Dorsselaer and F. Schuber, *J. Biol. Chem.*, 2005, **280**, 6479.
93 C. T. Hou, *Adv. Appl. Microbiol.*, 2000, **47**, 201.
94 M. Schottler and W. Boland, *Helv. Chim. Acta*, 1995, **78**, 847.
95 W. Yang, L. Dostal and J. P. N. Rosazza, *Appl. Environ. Microbiol.*, 1993, **59**, 281.
96 V. Pedrotta and B. Witholt, *J. Bacteriol.*, 1999, **181**, 3256.
97 R. Holtwick, H. Keweloh and F. Meinhardt, *Appl. Environ. Microbiol.*, 1999, **65**, 2644.
98 A. von Wallbrunn, H. H. Richnow, G. Neumann, F. Meinhardt and H. J. Heipieper, *J. Bacteriol.*, 2003, **185**, 1730.
99 H. J. Heipieper, G. Neumann, N. Kabelitz, M. Kastner and H. H. Richnow, *Appl. Microbiol. Biotechnol.*, 2004, **66**, 285.
100 P. E. Hughes, W. J. Hunter and S. B. Tove, *J. Biol. Chem.*, 1982, **257**, 3643.
101 J. L. van de Vossenberg and K. N. Joblin, *Lett. Appl. Microbiol.*, 2003, **37**, 424.
102 E. E. Mosley, G. L. Powell, M. B. Riley and T. C. Jenkins, *J. Lipid Res.*, 2002, **43**, 290.
103 F. R. Taylor, A. Kandutsch, A. K. Gayen, J. A. Nelson, S. S. Nelson, S. Phirwa and T. A. Spencer, *J. Biol. Chem.*, 1986, **261**, 15039.
104 C. Baumann, Diss. ETH # 9274, 1990.
105 J. S. Sinninghe Damste, M. Strous, W. I. Rijpstra, E. C. Hopmans, J. A. Geenevasen, A. C. van Duin, L. A. van Niftrik and M. S. Jetten, *Nature*, 2002, **419**, 708.
106 H. A. Boumann, E. C. Hopmans, I. van de Leemput, H. J. Op den Camp, J. van de Vossen-berg, M. Strous, M. S. Jetten, J. S. Sinninghe Damste and S. Schouten, *FEMS Microbiol. Lett.*, 2006, **258**, 297.
107 M. Strous, E. Pelletie, S. Mangenot, T. Rattei, A. Lehner, M. W. Taylor, M. Horn, H. Daims, D. Bartol-Mavel, P. Wincker, V. Barbe, N. Fonknechten, D. Vallenet, B. Segurens, C. Schenowitz-Truong, C. Medigue, A. Collingro, B. Snel, B. E. Dutilh, H. J. Op den Camp, C. van der Drift, I. Cirpus, K. T. van de Pas-Schoonen, H. R. Harhangi, L. van Niftrik, M. Schmid, J. Keltjens, J. van de Vossenberg, B. Kartal, H. Meier, D. Frishman, M. A. Huynen, H. W. Mewes, J. Weissenbach, M. S. Jetten, M. Wagner and D. Le Paslier, *Nature*, 2006, **440**, 790.
108 V. Mascitti and E. J. Corey, *J. Am. Chem. Soc.*, 2004, **126**, 15664.
109 V. Mascitti and E. J. Corey, *J. Am. Chem. Soc.*, 2006, **128**, 3118.
110 G. Zuber, M. Goldsmith, D. N. Beratan and P. Wipf, *Chirality*, 2005, **17**, 507.
111 L. Kunst and A. L. Samuels, *Prog. Lipid Res.*, 2003, **42**, 51.

112 E. B. Cahoon, J. A. Schnurr, E. A. Huffmann and R. E. Minto, *Plant J.*, 2003, **34**, 671.
113 R. Smith Goodloe and R. J. Light, *Biochim. Biophys. Acta*, 1982, **711**, 261.
114 M. Dennis and P. E. Kolattukudy, *Proc. Natl. Acad. Sci. U. S. A.*, 1992, **89**, 5306.
115 F. Schneider-Belhaddad and P. Kolattukudy, *Arch. Biochem. Biophys.*, 2000, **377**, 341.
116 J. Z. Shyadehi, D. C. Lamb, S. L. Kelly, D. E. Kelly, W. H. Schunck, J. N. Wright, D. Corina and M. Akhtar, *J. Biol. Chem.*, 1996, **271**, 12445.
117 M. Akhtar, P. Lee-Robichaud, M. E. Akhtar and J. N. Wright, *J. Steroid Biochem. Mol. Biol.*, 1997, **61**, 127.
118 J. R. Reed, D. Vanderwel, S. Choi, J. G. Pomonis, R. C. Reitz and G. J. Blomquist, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 10000.
119 Adapted from J. R. Reed, D. R. Quilici, G. J. Blomquist and R. C. Reitz, *Biochemistry*, 1995, **34**, 16221.
120 B. Jacob, *Nat. Prod. Rep.*, 2006, **23**, 851.
121 P. A. Frey and S. J. Booker, *Adv. Protein Chem.*, 2001, **58**, 1. Review.
122 D. Layer, W. Heinz, D. Jahn and W. D. Schubert, *Curr. Opin. Chem. Biol.*, 2004, **8**, 468.
123 R. J. Parry, *Tetrahedron*, 1983, **39**, 1215.
124 A. Marquet, *Curr. Opin. Chem. Biol.*, 2001, **5**, 541.
125 F. Berkovitch, Y. Nicolet, J. T. Wan, J. T. Jarrett and C. L. Drennan, *Science*, 2004, **303**, 76.
126 Y. Nicolet and C. L. Drennan, *Nucleic Acids Res.*, 2004, **32**, 4015.
127 P. Genix, Diss. ETH # 7882, 1985.
128 D. N. Harpp and J. Gleason, *J. Am. Chem. Soc.*, 1971, **93**, 2437.
129 A. Gauthier, Diss ETH # 6583, 1980.
130 S. Shapiro, J. U. Piper and E. Caspi, *J. Am. Chem. Soc.*, 1982, **104**, 2301.
131 M. A. Hayden, I. Y. Huang, G. Iliopoulos, M. Orozco and G. W. Ashley, *Biochemistry*, 1993, **32**, 3778.
132 R. Miller, R. W. Busby, S. W. Jordan, J. Cheek, T. F. Henshaw, G. W. Ashley, J. B. Broderick, J. E. Cronan, Jr. and M. A. Marletta, *Biochemistry*, 2000, **39**, 15166.
133 S. J. Booker, *Chem. Biol.*, 2004, **11**, 10.
134 R. M. Cicchillo, K. H. Lee, C. Baleanu-Gogonea, N. M. Nesbitt, C. Krebs and S. J. Booker, *Biochemistry*, 2004, **43**, 11770.
135 R. M. Cicchillo, D. F. Iwig, A. D. Jones, N. M. Nesbitt, C. Baleanu-Gogonea, M. G. Souder, L. Tu and S. J. Booker, *Biochemistry*, 2004, **43**, 6378.
136 J. E. Cronan, X. Zhao and Y. Jiang, *Adv. Microb. Physiol.*, 2005, **50**, 103.
137 M. Cicchillo and S. J. Booker, *J. Am. Chem. Soc.*, 2005, **127**, 2860.
138 F. Marti, Diss. ETH # 7236, 1983.
139 I. Sanyal, G. Cohen and D. H. Flint, *Biochemistry*, 1994, **33**, 3625.
140 G. N. Jameson, M. M. Cosper, H. L. Hernandez, M. K. Johnson and B. H. Huynh, *Biochemistry*, 2004, **43**, 2022.
141 B. T. Bui, D. Florentin, F. Fournier, O. Ploux, A. Mejean and A. Marquet, *FEBS Lett.*, 1998, **440**, 226.
142 M. Lotierzo, B. Tse Sum Bui, D. Florentin, F. Escalettes and A. Marquet, *Biochem. Soc. Trans.*, 2005, **33**, 820.
143 M. Lotierzo, E. Raux, B. Tse Sum Bui, N. Goasdoue, F. Libot, D. Florentin, M. J. Warren and A. Marquet, *Biochemistry*, 2006, **45**, 12274.
144 R. B. Broach and J. T. Jarrett, *Biochemistry*, 2006, **45**, 14166.
145 J. T. Groves, *J. Inorg. Biochem.*, 2006, **100**, 434.
146 M. Newcomb, R. Zhang, R. E. Chandrasena, J. A. Halgrimson, J. H. Horner, T. M. Makris and S. G. Sligar, *J. Am. Chem. Soc.*, 2006, **128**, 4580.
147 M. Newcomb and R. E. Chandrasena, *Biochem. Biophys. Res. Commun.*, 2005, **338**, 394.
148 S. Shaik, D. Kumar, S. P. de Visser, A. Altun and W. Thiel, *Chem. Rev.*, 2005, **105**, 2279.
149 J. Shanklin, C. Achim, H. Schmidt, B. G. Fox and E. Munck, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 2981.
150 H. F. Hsu, L. Que and J. Shanklin, *J. Inorg. Biochem.*, 1999, **74**, 168.
151 J. Shanklin and E. Whittle, *FEBS Lett.*, 2003, **545**, 188.
152 J. B. van Beilen, T. H. Smits, F. F. Roos, T. Brunner, S. B. Balada, M. Rothlisberger and B. Witholt, *J. Bacteriol.*, 2005, **187**, 85.
153 H. Fu, M. Newcomb and C. H. Wong, *J. Am. Chem. Soc.*, 1991, **113**, 5878.
154 R. N. Austin, H. K. Chang, G. J. Zylstra and J. T. Groves, *J. Am. Chem. Soc.*, 2000, **122**, 11747.
155 E. Caspi, J. U. Piper and S. Shapiro, *J. Chem. Soc., Chem. Commun.*, 1981, 76.
156 E. G. Funhoff, U. Bauer, I. Garcia-Rubio, B. Witholt and J. B. van Beilen, *J. Bacteriol.*, 2006, **188**, 5220.
157 F. P. Guengerich, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 13565.
158 J. K. Yano, M. R. Wester, G. A. Schoch, K. J. Griffin, C. S. Stout and E. F. Johnson, *J. Biol. Chem.*, 2004, **279**, 38091.
159 P. A. Williams, J. Cosme, D. M. Vinkovic, A. Ward, H. C. Angove, P. J. Day, C. Vonrhein, I. J. Tickle and H. Jhoti, *Science*, 2004, **305**, 683.
160 C. R. Otey, M. Landwehr, J. B. Endelman, K. Hiraga, J. D. Bloom and F. H. Arnold, *PLoS Biol.*, 2006, **4**, 112.
161 M. J. Cryle and J. J. De Voss, *Angew. Chem., Int. Ed.*, 2006, **45**, 8221.
162 X. He, M. J. Cryle, J. J. De Voss and P. R. de Montellano, *J. Biol. Chem.*, 2005, **280**, 22697.
163 M. J. Cryle, P. R. Ortiz de Montellano and J. J. De Voss, *J. Org. Chem.*, 2005, **70**, 2455.
164 P. R. Ortiz de Montellano and J. J. De Voss, *Nat. Prod. Rep.*, 2002, **19**, 477.
165 B. G. Fox, J. Shanklin, J. Y. Ay, T. M. Loehr and J. Sanders-Loehr, *Biochemistry*, 1994, **33**, 12776.
166 J. Shanklin, E. Whittle and B. G. Fox, *Biochemistry*, 1994, **33**, 12787.
167 W. C. Man, M. Miyazaki, K. Chu and J. M. Ntambi, *J. Biol. Chem.*, 2006, **281**, 1251.
168 P. H. Buist and B. Behrouzian, *J. Am. Chem. Soc.*, 1996, **118**, 6295.
169 B. Phetsuksiri, M. Jackson, H. Scherman, M. McNeil, G. S. Besra, A. R. Baulard, R. A. Slayden, A. E. DeBarber, C. E. Barry, 3rd, M. S. Baird, D. C. Crick and P. J. Brennan, *J. Biol. Chem.*, 2003, **278**, 53123.
170 Y. Chang and B. G. Fox, *Biochemistry*, 2006, **45**, 13476.
171 D. H. Dyer, K. S. Lyle, I. Rayment and B. G. Fox, *Protein Sci.*, 2005, **14**, 1508.
172 T. Morikawa, M. Mizutani, N. Aoki, B. Watanabe, H. Saga, S. Saito, A. Oikawa, H. Suzuki, N. Sakurai, D. Shibata, A. Wadano, K. Sakata and D. Ohta, *Plant Cell*, 2006, **18**, 1008.
173 C. Kumar, S. P. De Visser and S. Shaik, *J. Am. Chem. Soc.*, 2004, **126**, 5072.
174 J. A. Broadwater, E. Whittle and J. Shanklin, *J. Biol. Chem.*, 2002, **277**, 15613.
175 M. Serra, L. T. Gauthier, G. Fabrias and P. H. Buist, *Insect Biochem. Mol. Biol.*, 2006, **36**, 822.
176 D. W. Reed, D. R. Polichuk, P. H. Buist, S. J. Ambrose, R. J. Sasata, C. K. Savile, A. R. Ross and P. S. Covello, *J. Am. Chem. Soc.*, 2003, **125**, 10635.
177 J. L. Abad, S. Rodriguez, F. Camps and G. Fabrias, *J. Org. Chem.*, 2006, **71**, 7558.
178 A. S. Carlsson, S. Thomaeus, M. Hamberg and S. Stymne, *Eur. J. Biochem.*, 2004, **271**, 2991.
179 A. L. K. Shi Shun and R. R. Tykwinski, *Angew. Chem., Int. Ed.*, 2006, **45**, 1034.
180 A. S. Ratnayake and T. Hemscheidt, *Org. Lett.*, 2002, **4**, 4667.
181 H. Yun, T. C. Chou, H. Dong, Y. Tian, Y. M. Li and S. J. Danishefsky, *J. Org. Chem.*, 2005, **70**, 10375.
182 S. Raduner, A. Majewska, J. Chen, X. Xie, J. Hamon, B. Faller, K. Altmann and J. Gertsch, *J. Biol. Chem.*, 2006, **281**, 14192.
183 C. H. Fawcett, D. M. Spencer, R. L. Wain, A. G. Fallis, E. R. Jones, M. Le Quan, C. B. Page, V. Thaller, D. C. Shubrook and P. M. Whitham, *J. Chem. Soc. C*, 1968, **19**, 2455.
184 D. C. Braddock, *Org. Lett.*, 2006, **8**, 6055.
185 Adapted from A. Murai, in *Comprehensive Natural Products Chemistry, Volume 1: Polyketides and Other Secondary Metabolites Including Fatty Acids and Their Derivatives*, ed. D. H. R. Barton, O. Meth-Cohn, K. Nakanishi and Y. Sankawa (volume editor), Elsevier, Oxford, 1999, p. 303–324.
186 M. Lee, M. Lenman, A. Banas, M. Bafor, S. Singh, M. Schweizer, R. Nilsson, C. Liljenberg, A. Dahlqvist, P. O. Gummeson, S. Sjodahl, A. Green and S. Stymne, *Science*, 1998, **280**, 915.
187 O. Sayanova, R. Haslam, I. Guschina, D. Lloyd, W. W. Christie, J. L. Harwood and J. A. Napier, *J. Biol. Chem.*, 2006, **281**, 36533.
188 W. L. Smith and R. C. Murphy, in, *Biochemistry of Lipids, Lipoproteins and Membranes*, 4th edn, ed. D. E. Vance and J. E. Vance, Elsevier, Amsterdam, 2002, pp. 341–371.
189 W. H. Gerwick, in *Comprehensive Natural Products Chemistry, Volume 1: Polyketides and Other Secondary Metabolites Including Fatty Acids and Their Derivatives*, ed. D. H. R. Barton, O. Meth-Cohn,

K. Nakanishi and Y. Sankawa (volume editor), Elsevier, Oxford, 1999, p. 207–250.
190 K. Schwarz, M. Anton and H. Kuhn, *Adv. Exp. Med. Biol.*, 2002, **507**, 55.
191 A. Liavonchanka and I. Feussner, *J. Plant Physiol.*, 2006, **163**, 348.
192 C. M. McGinley and W. A. van der Donk, *Chem. Commun.*, 2003, **7**, 2843.
193 C. R. Goldsmith, R. T. Jonas and T. D. Stack, *J. Am. Chem. Soc.*, 2002, **124**, 83.
194 C. R. Goldsmith and T. D. Stack, *Inorg. Chem.*, 2006, **45**, 6048.
195 N. Lehnert and E. I. Solomon, *J. Biol. Inorg. Chem.*, 2003, **8**, 294.
196 J. P. Klinman, *Biochim. Biophys. Acta*, 2006, **1757**, 981.
197 M. J. Knapp and J. P. Klinman, *Biochemistry*, 2003, **42**, 11466.
198 G. Pohnert, *ChemBioChem*, 2005, **6**, 946.
199 I. Feussner and C. Wasternack, *Annu. Rev. Plant Biol.*, 2002, **53**, 275.
200 E. Hofmann, P. Zerbe and F. Schaller, *Plant Cell.*, 2006, **18**, 3201.
201 W. C. Song, S. W. Baertschi, W. E. Boeglin, T. M. Harris and A. R. Brash, *J. Biol. Chem.*, 1993, **268**, 6293.
202 A. Itoh and G. A. Howe, *J. Biol. Chem.*, 2001, **276**, 3620.
203 V. Ullrich, *Arch. Biochem. Biophys.*, 2003, **409**, 45.
204 M. Hamberg, *FEBS J.*, 2005, **272**, 736.
205 F. H. Vaillancourt, E. Yeh, D. A. Vosburg, S. E. O'Connor and C. T. Walsh, *Nature*, 2005, **436**, 1191.
206 F. H. Vaillancour, E. Yeh, D. A. Vosburg, S. Garneau-Tsodikova and C. T. Walsh, *Chem. Rev.*, 2006, **106**, 3364.
207 C. Sanchez, L. Zhu, A. F. Brana, A. P. Salas, J. Rohr, C. Mendez and J. A. Salas, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 461.
208 A. Yarnell, *Chem. Eng. News*, 2006, **84**, 12.
209 H. Deng, S. L. Cobb, A. R. McEwan, R. P. McGlinchey, J. H. Naismith, D. O'Hagan, D. A. Robinson and J. B. Spencer, *Angew. Chem., Int. Ed.*, 2006, **45**, 759.
210 T. Wichard and G. Pohnert, *J. Am. Chem. Soc.*, 2006, **128**, 7114.