Biosynthesis and Metabolism of Cyclopropane Rings in Natural Compounds

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I. Introduction

Cyclopropane subunits occur in many natural products of secondary metabolism.[1] The majority of cyclopropane-containing natural compounds have been isolated from plants, fungi, or microorganisms. Many show biological activity and may serve as potential drug leads or provide new ideas for the study of enzyme mechanisms. Examples include 1-aminocyclopropane-1-carboxylic acid (ACC) as the general precursor of the plant hormone ethylene, coronatin as a strong elicitor of stress response in plants, pyrethroids as insecticides,[2] ptaquilosides causing, e.g., carcinomas,[3,4] and curacin A and D[5] and CC-1065[6−11] as drug leads. As is commonly the case in natural products chemistry, the real significance of these compounds in their natural context is often less well known than their biological properties on human defined targets, which are commonly discovered in general screening programs. Usually, some correlation between natural significance and application by humans can be established later (e.g., with coronamic acid). Sometimes, however, observation of natural phenomena precedes isolation and/or application, as in the case of the pyrethroids as insecticides. Thousands of natural compounds and their derivatives carrying a cyclopropane group have been synthesized and described in the literature. Their isolation and chemical synthesis, however, are the subject of a corresponding article that we will publish in the future.[12] In the present review, we will focus on the principal mechanisms of the biosynthesis and metabolism of cyclopropane-containing natural products and selected related compounds. Our main concern here will be the formation, transformation, and degradation of the cyclopropane moiety itself. The metabolism of non-natural cyclopropane-containing compounds—applied, e.g., as biological tools, metabolic probes, or inhibitors—is not included in this article.

Cyclopropanes can serve natural demands in many ways. In compounds where they form a chemically stable moiety, they can simply be a space element of a certain dimension and lipophilicity with an orientation or position differing from that of closely related open-chain moieties. In such a comparison, a cyclopropyl is a little smaller than an isopropyl unit (cf. also the artificial drugs naltrexone and the blockbuster antibiotic ciprofloxacin), spirocyclopropyl is a little smaller than a geminal dimethyl group, and an annulated cyclopropane is smaller and its exo-carbon

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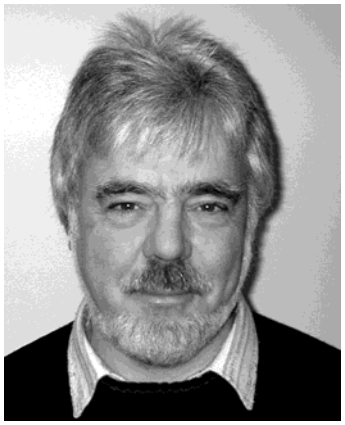
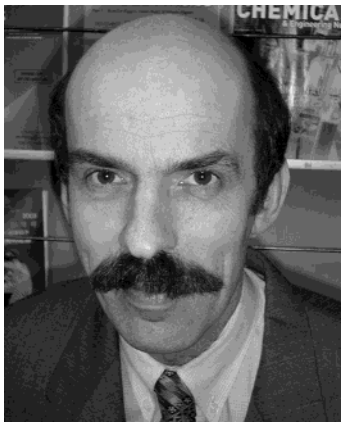
Ludger Wessjohann studied chemistry in Hamburg, Germany, and Southampton, UK, and received his degree from the University of Hamburg (M.Sc. 1987). During his Ph.D. studies, he worked as a visiting scientist with Professor Lars Skattebøl in Oslo, Norway (1987–1988). His Ph.D. thesis (1990, with Professor de Meijere) covered synthetic approaches to small-ring amino acids and building blocks. In 1990, he became a lecturer and advisor for a German government organization at the Universidade Federal de Santa Maria, Brazil, where he also was a visiting professor in 1993 and 1995. From late 1990 to 1992, he was a postdoctoral fellow of the Alexander von Humboldt foundation with Professor Paul Wender at Stanford University, California, where he worked on the total synthesis of Taxol. Upon returning to Germany, he became an assistant professor at the Ludwig-Maximilians-Universität München and received his habilitation in July 1998. In June 1998, he became full professor of bioorganic chemistry at the Vrije Universiteit Amsterdam, The Netherlands. In 2000, he accepted a call to the Institute of Plant Biochemistry in Halle, Germany, a government research institute of the Leibniz association, where he is currently the director of the department of bioorganic chemistry. Professor Wessjohann in parallel holds the chair of natural product chemistry at the Martin-Luther-University Halle-Wittenberg. His research interests include the development of new synthetic methods—especially of selenium- and chromium-mediated reactions—total and combinatorial synthesis, medicinal chemistry, biocatalysis, and natural products chemistry.



Wolfgang Brandt received his B.Sc. and Ph.D. from the University of Halle, Germany, in 1979 and 1981, respectively. His postdoctoral research was done at the University of Halle (1981–1984) and later at the Institute of Neurobiology and Brain Research of the Academy of Science of the former German Democratic Republic in Magdeburg (1985–1986). In 1986, he became head of the research group "Molecular Modelling–Drug Design" at the Department of Biochemistry–Biotechnology of the University of Halle. In 1990, he was a visiting scientist (three months) at the Pharmaceutical Institute of the Free University Berlin, Germany (Prof. Dr. H.-D. Höltje), and in 1992, he was a visiting scientist (six months) at the Clinical Research Institute of Montreal, Canada (Prof. Dr. P. W. Schiller). In 1997, he received his habilitation in the research field of molecular modeling and drug design from the University of Halle, Germany. Since 2001, he has been head of the research group "Computational Chemistry and Molecular Modeling" at the Leibniz Institute of Plant Biochemistry in the department of bioorganic chemistry in Halle. He has published 75 papers in refereed scientific journals and made about 40 announcements at international scientific conferences.

is twisted sideways compared to a methyl group. More importantly, cyclopropanes can serve as rigid structural elements, causing reduced conformational flexibility in their annulated, spiro, or di- to oligo-substituted form. Examples in which this can lead to interesting properties are cyclopropane amino acids and peptides with these.[13−22] In addition to rigidification, the angles are locked and deviate from the standard tetrahedral angle. Thus, angles are reduced within the ring, and substituent angles are enlarged in spiro or 1,1-disubstituted cyclopropanes (to about 118° versus 109.5° in saturated systems, Scheme 1). 1,2-Disubstituted cyclopropanes can serve as a rigid two-carbon cis or trans connections comparable to a double bond, but with different bond lengths (about 1.51 Å versus 1.34 Å for a double bond), bond angles (119° in alkenes versus 123° in cyclopropanes), and dihedral angles (about 141° for *trans*-1,2-dimethylcyclopropane in comparison to 180° for *trans*-1,2-dimethylethylene, Scheme 1). In addition to steric effects, the stereoelectronic and electronic properties of cyclopropane bonds, with their stronger s-orbital influence (sp^2-likeness) and banana shape, differ from simple sp^3 bonds and also affect the neighborhood.[23]

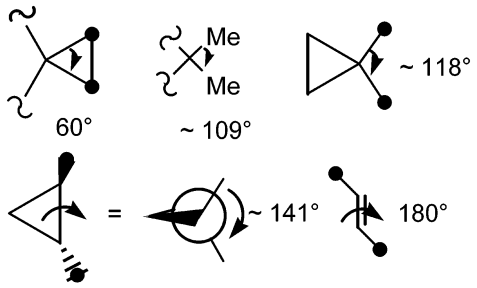
In other cases, the strained cyclopropane moiety represents a labile element, with its stored strain energy as a driving force (some 115 kJ mol^{-1}, depending significantly on the substitution pattern).



Thies Thiemann has studied chemistry at the Universities of Hamburg and Tübingen. He received his M.Sc. (1986) and his D.Sc. from the University of Hamburg (1992). A postdoctoral stay at the Institute of Advanced Material Study, Kyushu University, Japan, was followed by an Assistant Professorship at the same university. He has been a visiting scientist at the University of Coimbra (Prof. Dr. A. S. Campos Neves) and at the Université Libre de Bruxelles (Prof. Dr. C. Braekman-Danheux and Prof. Dr. A. Fontana) within CECA and HCM projects. Since 1997, he has been Associate Professor at Kyushu University, Fukuoka, Japan. His research interests include heterocyclic chemistry, steroidal chemistry, and "small-ring" chemistry. He has published about 70 papers in these fields and has written two book chapters.

Cyclopropanes can serve as high-energy intermediates in metabolism (e.g., presqualene), as storage elements to release energy-rich compounds (e.g., ethylene from ACC oxidation), or as trigger components to provide a driving force and ensure irreversibility in mechanism-based inhibition (e.g., CC-1065).[6,9−11]

Scheme 1



The structures found in nature can be classified on the basis of their natural origin, their structural framework, or the chemical mechanisms of the formation or cleavage of the cyclopropane ring. Representative structures of cyclopropane-containing compounds are found mostly in the natural product classes of the terpenoids and steroids, amino acids and alkaloids, fatty acids, and polyketides, among others. With regard to the mechanism of the biosynthesis pathway, the cyclopropane group can be formed via cationic intermediates (e.g., from diphosphate abstraction) reacting with homoallylic bonds (Scheme 2a, cf. also Scheme 3), with double bonds (Scheme 2b), or with suitably oriented α-methyl groups (Scheme 2c), by allyl cation-to-cyclopropyl cation rearrangement (Scheme 2d), or by addition of a double bond to the methyl cation equivalent *S*-adenosylmethionine (SAM, Scheme 2e). Radical mechanisms and related processes can also produce cyclopropanes, e.g., peroxide decomposition (Scheme 2f), transition metal catalysis (Scheme 2g), photochemical excitation (Scheme 2h), or a redox mechanism mediated by NAD(P)/NAD(P)H (Scheme 2i). The latter may also be considered an anionic (hydride-transfer) mechanism and thus may be grouped with S_Ni-anionic ring closures to natural cyclopropanes (Scheme 2j, types I and II). It should be remarked, however, that several of the mechanisms presented in the literature are speculative or based on weak evidence. Only in a few cases were in-depth studies regarding the cyclopropane formation done, e.g., by detailed labeling experiments or enzyme or inhibitor studies. In some cases, we included our own hypotheses for possible biosynthetic mechanisms. We have done this in cases where the new routes suggested conform with experimental evidence or chemical wisdom. In other cases, very similar mechanisms have been proven for related compounds but were not yet considered in the case discussed, or we saw a need for a counter-hypothesis to a published one to trigger further studies.

II. Biosynthesis of the Cyclopropane Rings

An excellent overview of biosynthetic pathways and metabolisms of cyclopropane-containing natural products was written by Liu and Walsh in 1987.[1] Data already contained in their review will be summarized only briefly and to the extent necessary to give a complete picture under the different aspect and evaluation presented here. These older data are accompanied by a more extensive coverage of results which have appeared in the literature since 1986. Thus, for a complete listing of references before 1987, the aforementioned review should be consulted. For the synthesis of cyclobutane rings in natural products, which are to some extent biogenetically related through the transient homoallyl cations (e.g., Schemes 2a and 3), see a dedicated review in *Houben-Weyl*.[25]

A. Rearrangement of Carbon Skeletons via Cationic Intermediates

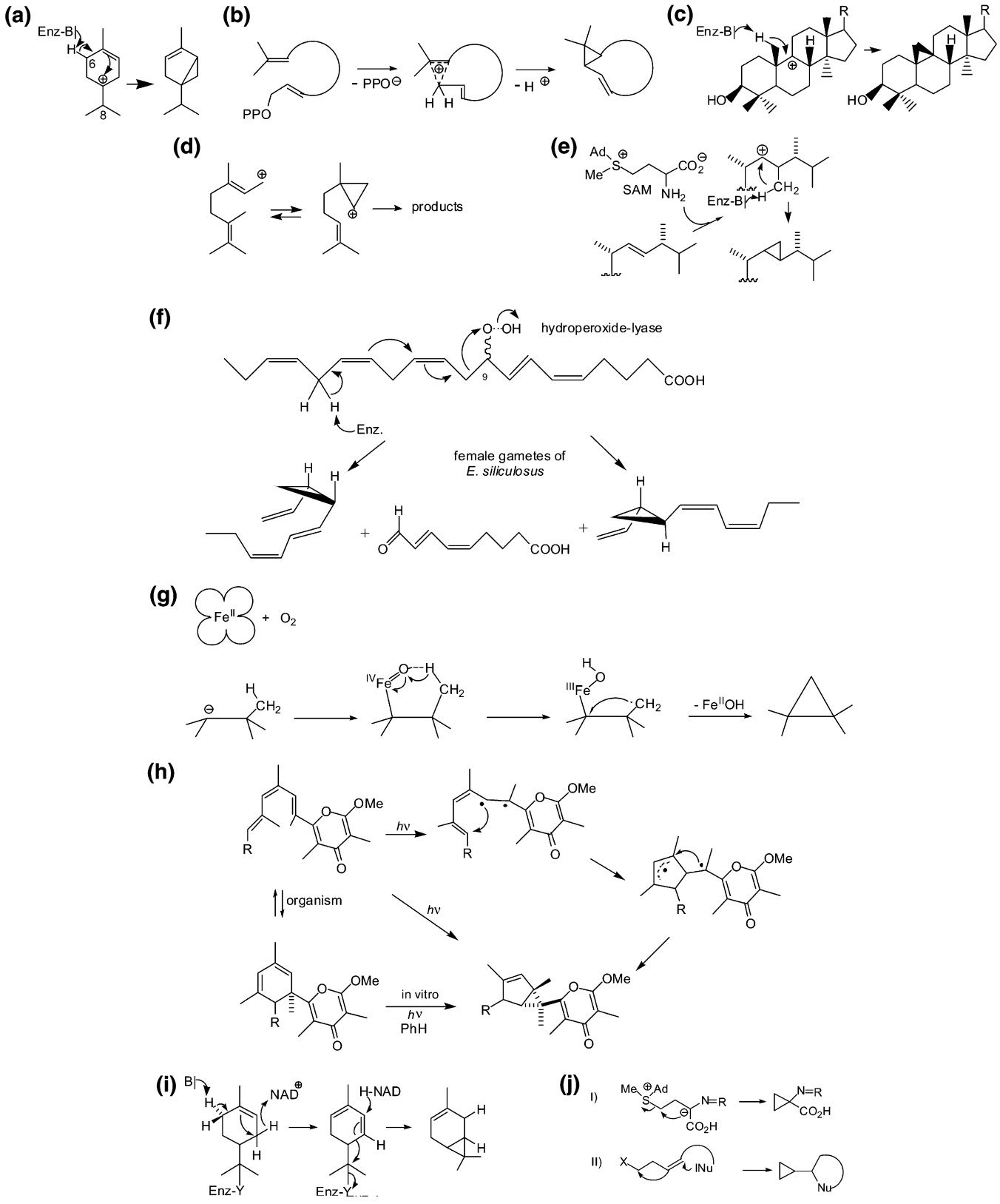
The general basic principle of the cyclopropane ring formations covered in this section is the electrophilic attack of a carbenium ion on a homoconjugated double bond, an allylic double bond, a double bond, or a methyl group (cf. Scheme 2a−d). With the exeption of the SAM-dependent cyclopropanation of unsaturated fatty acids, most cyclopropanations involving cationic intermediates take place with isoprenoids as substrates, i.e., terpenoids or steroids mostly.

In linear isoprenoids, the formation of the cation is usually initiated by the cleavage of a diphosphate group (pyrophosphate, OPP). Most commonly, isoprenoid allylic diphosphates are activated by terpene or sterol cyclases with the help of Mg^{2+} cations, or rarely Mn^{2+} or Zn^{2+}, to form an allyl cation intermediate which cyclizes. The general mechanism of terpene cyclases has been elucidated, e.g., in the works of Croteau[26] and Poulter.[27−31] It should be mentioned that the involvement of free cations in an absolute sense is highly unlikely. Instead, an ion pair with the counterion stabilized by enzymatic interaction is a more likely intermediate. The terpene or steroid cyclases arrange the prenyl chains (**1**, Scheme 4) in such a way that the desired nucleophilic double bond is brought into the proximity of the developing cationic charge in order to form a defined cyclic intermediate. This, in many cases, is followed by further (internal) attack of the new cation on other proximal double bonds, by rearrangements of the carbon skeleton, or by proton shifts. During these additions and rearrangements, small rings are commonly formed, sometimes as intermediates, and often as remaining structural elements (cf., e.g., Scheme 13). Within the small rings, cyclopropanes are immensely favored over cyclobutanes, as would be expected from both the theory of ring-closing kinetics and the basic isoprenoid structure, with allylic (i.e., 1,3-) elements separated by two carbon bridges (Scheme 4).

Finally, the cationic (chain) reaction is terminated either by proton loss to yield a double bond or by nucleophile addition, mostly of water, to form alcohols (cf., e.g., Scheme 6), or in some cases even by readdition of diphosphate. The latter is possible only in thermodynamically favorable cyclizations, i.e., rarely if medium- or small-ring formation is required, where the loss of an inorganic diphosphate (not two phosphates) delivers the necessary energy. Other termination possibilities, e.g., redox reactions, are possible and have been suggested in some cases (cf., e.g., Scheme 2f,i).

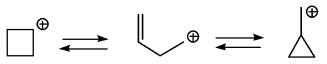
Cyclopropanes can also be formed within already-cyclized terpenoids if the necessary energy is available. This energy can be supplied by a leaving group

Scheme 2. The Most Common Principal Mechanisms of Cyclopropane Formation in Nature[a]

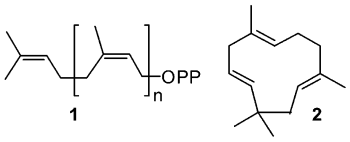


[a] (a) Reaction of an intermediate cation with a homoconjugated double bond (homoallyl cation ring closure). (b) Reaction of an intramolecular (allyl) cation with a double bond to form a protonated cyclopropane species and its subsequent deprotonation. (c) Reaction of an intermediate cation with an enzyme-activated α-methyl group. (d) Rearrangement of an allyl cation to a cyclopropyl cation (suggested).[24] (e) Reaction of an intermediate cation with a neighboring methyl group generated by a methyl transfer from *S*-adenosylmethionine (SAM) to a precursor double bond acting as a nucleophile. (f) Reaction of a radical intermediate with a homoconjugated double bond (derived from a peroxide fragmentation). (g) Transition metal-assisted radical cyclization. (h) Photochemically driven rearrangements (a mechanism newly proposed by us, see below). (i) Redox mechanism supported by NAD(P) [H• or H⁻ transfer mechanism]. (j) Internal nucleophilic substitution (S_Ni).

Scheme 3. The Cyclobutyl−Homoallyl−Cyclopropylmethyl Cation Interchange



Scheme 4



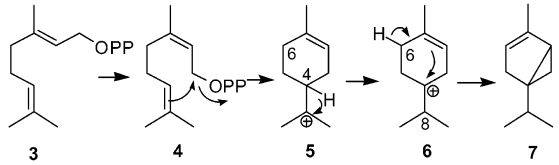
(OPP or other) or, more commonly, through ring strain; examples of the latter can be found in formations involving cyclobutane rearrangement (cf. Scheme 9, illudin) or transannular strain release. An important and well-studied example of a medium-sized precursor for many cyclopropane-containing sesquiterpenoids is humulene (**2**, Scheme 4, vide infra). In general, small- and medium-sized rings are frequently formed in nature, and five- and six-membered rings are a little less dominant than in synthetic compounds. Also, probably not all terpene cyclizations or rearrangements are ruled by enzymatic activity, or at least the conformational bias inflicted by the enzymes during cyclization does not always appear to be strict, as sometimes isomeric products are formed in these processes.

In steroids and fatty acids, additions of cations (H^+ from the enzyme or Me^+ from SAM as cofactor, vide infra) to double bonds play a major role in the cation precursor formation, rather than elimination of a leaving group. Also, the termination of the process often proceeds by deprotonation at the β-carbon under cyclopropane bond formation (cf. Scheme 2c,e) rather than the other processes described previously for terpenoids.

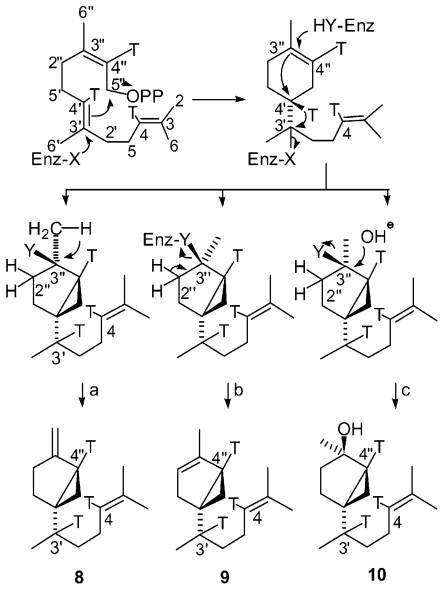
1. Cyclizations of Allyl and Homoallyl Cations

Cyclizations of homoallylic cations to cyclopropanes (cf. Scheme 2a) are commonly observed in isoprenoid rearrangements (cf. Scheme 41). Bicyclic monoterpenes are present in many plants. Among the members of this family are thujanes, such as α-thujene, 3-thujone, 3-thujanol, and α-sabinene. The formation of the cyclopropyl group in 3-thujone and other members of this family starts from a cyclization of the acyclic C_{10} isoprenyl precursor geranyl diphosphate (**3**, Scheme 5). The cleavage of the diphosphate group (**4**) is accompanied by cyclization and the formation of the α-terpinyl cation (**5**). A subsequent 1,2-hydride shift from C4 and attack of the C4 cation on the double bond result in the formation of a cyclopropane ring. After loss of the α-proton to a C(1,6) double bond, finally α-thujene (**7**) is formed (Scheme 5).

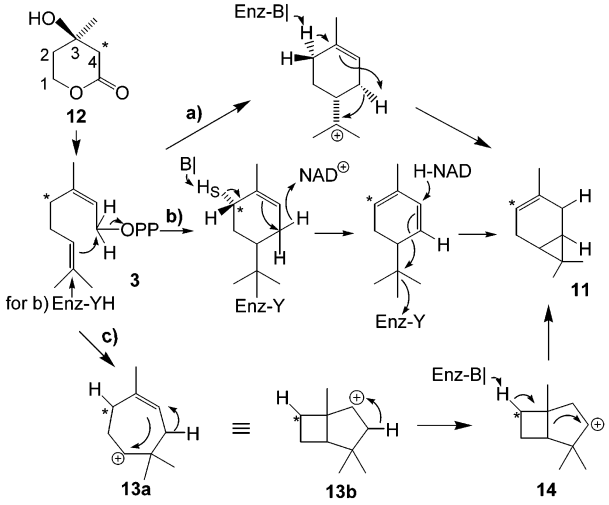
Scheme 5



Scheme 6



Scheme 7

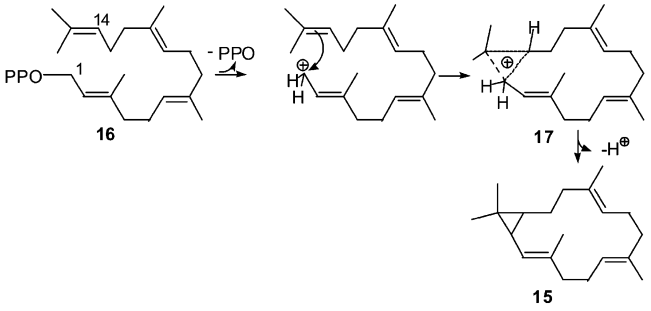


This principal mechanism and the involvement of the homoconjugated double bond in the hypothetical monocyclic α-terpinyl cation (**6**) are supported by labeling studies in vivo of 3-thujone, sabinene, and other terpenoids derived from mevalonic acid.[32−35] For a more detailed discussion of the mechanisms and the possible contribution of enzymes, see the review by Liu and Walsh.[1]

Sharma et al. proved through tritium labels (T) that the sesquithujane skeleton (**8**−**10**) is also formed by a 1,2-hydrogen shift (Scheme 6).[36]

As an alternative to the intracyclic mechanisms shown in Scheme 5, the geranyl cation can be stabilized by formation of α-car-3-ene (**11**, Scheme 7). However, it could be shown that in this case it is not a simple 1,3-eliminiation that occurs but a double bond migration. This was proven by degradation studies of α-car-3-ene (**11**) derived from labeled mevalonolactone (**12**, asterisk indicates ^{14}C-labeled carbon).[37] Three alternative mechanisms that could explain the experimental results are discussed below, one of which is presented here for the first time.[1,24] Akhila and Banthorpe[24] suggested a concerted for-

Scheme 8



mation of the carene skeleton involving a proton migration (Scheme 7a). The second mechanism suggested by these authors involves a similar removal of two 1,4-allylic hydrogens, this time mediated by a sequential oxidation/reduction with participation of NAD(P)/NAD(P)H as a hydride shuttle (Scheme 7b).[1] We suggest a third possible mechanism (Scheme 7c), where a cycloheptenyl cation intermediate **13a** is formed (cf. also Scheme 10) which may be stabilized as a transannular nonclassical cation. In another form, this can also be written as bicyclo[3.2.0]-heptanyl cation **13b**. A 1,2-hydride shift in **13b** to give **14** would be followed by a cyclobutane opening to give car-3-ene (**11**) in a mechanism similar to the one discussed for illudin formation (cf. Scheme 9). Several compounds with the same carbon skeleton as the reactive intermediates **13b** and **14** have been isolated from natural sources, e.g., bourbonene, spatadiene, kelsoene, lintenone, tenerol, and others.

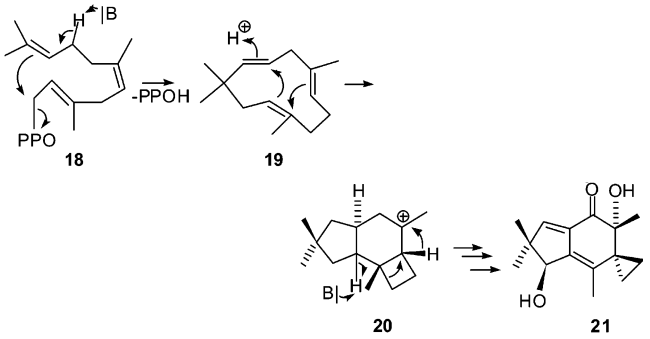
Casbene (**15**, Scheme 8) initially was isolated from castor beans (*Ricinus communis*).[38,39] Here the cyclopropane ring is formed via a nonclassical carbocation (a corner-protonated cyclopropane) formed from geranylgeranyl diphosphate (**16**, Scheme 8).

After cleavage of the diphosphate, the terminal cation interacts with the C(14,15) double bond, which leads to a macrocyclic ring system **17** with a protonated cyclopropane ring.[39,40] Finally, a proton is removed, and casbene (**15**) is formed as a stable compound. Moreover, casbene has also been discussed as an intermediate in the biosynthesis of polycyclic diterpenes, such as lathyrane, tigliane, and ingenane.[40,41]

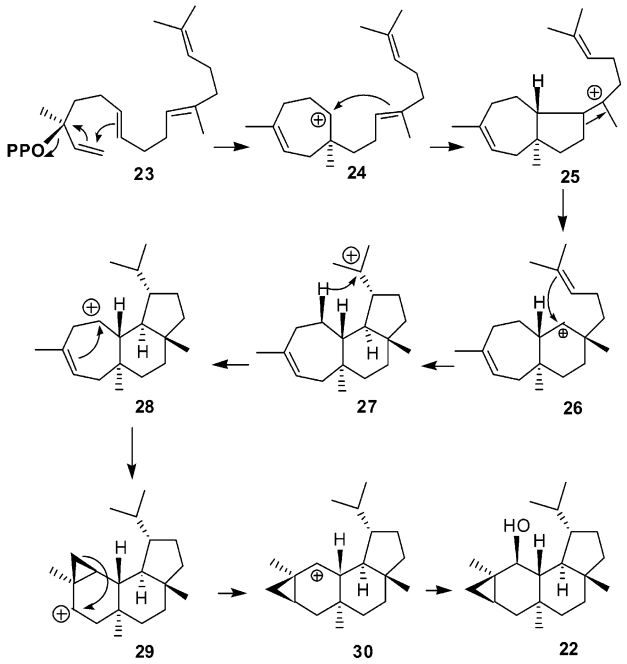
In a study reported by Pattenden and Smithies,[42] the mechanism of cyclopropane ring opening in casbene (**15**) was investigated. Using several radical-mediated reactions with casbene, they found a number of products which are in agreement with compounds found as metabolites, such as those from the cembrane family. The detailed mechanism, however, is not yet clear. The participation of a "casbene synthetase", which needs a divalent cation such as magnesium, is also discussed in the biosynthetic pathway of casbene.[43]

Illudins have been isolated from the bioluminescent mushrooms *Clitocybe illudens* and *Lampteromyces japonicus*.[44−46] In principle, the mechanism of formation of the rings is identical to those described previously. The precursor is farnesyl diphosphate (**18**) instead of geranylgeranyl diphosphate, and a humulene ring system (**19**) is formed in the first step, with concomitant loss of the diphosphate (Scheme 9).

Scheme 9



Scheme 10

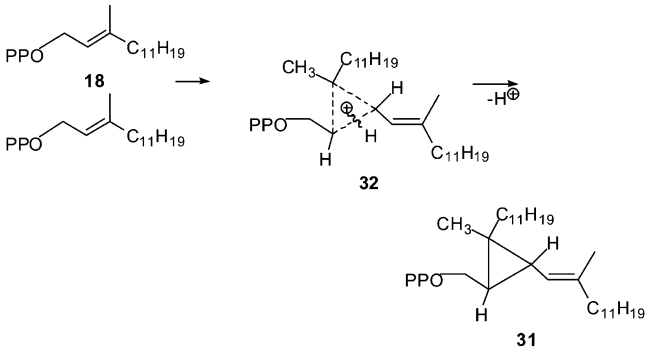


Rearrangement of the ring system, with intermediate loss of a proton and reprotonation, gives the protoilludyl cation (**20**), which rearranges to the illudene skeleton by the base-supported rearrangement depicted in Scheme 9. Finally, oxidations lead to illudin M (**21**) and other natural illudins. The detailed stereochemical pathway has been analyzed by Bradshaw et al.,[47] Cane and Nachbar,[48] and Shirahama et al.[49] and was also discussed by Liu and Walsh.[1]

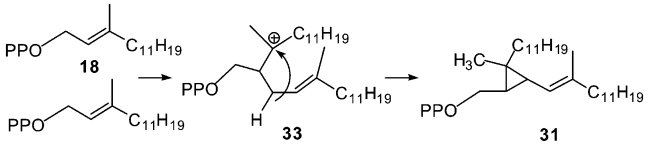
Verrucosanes form a family of tetracyclic diterpenes with a cyclopropane. In the green phototropic eubacterium *Chlorflexus aurantiacus*, the formation of verrucosan-2β-ol (**22**) was observed by Rieder et al., and its biosynthetic pathway was extensively investigated.[50] They demonstrated that the compound is synthesized via the mevalonate pathway.

Scheme 10 shows the suggested mechanism, which starts with the removal of diphosphate from geranyllinalyl diphosphate (**23**), which leads first to a cationic monocyclic (**24**) and then to a bicyclic intermediate (**25**). The latter undergoes a 1,2-carbon shift to **26**, which forms the tricyclic ring system **27**. Subsequently, the positive charge is stabilized by a 1,5-hydride transfer to give **28**. Interaction of the cation with the homoallylic double bond leads to the forma-

Scheme 11



Scheme 12



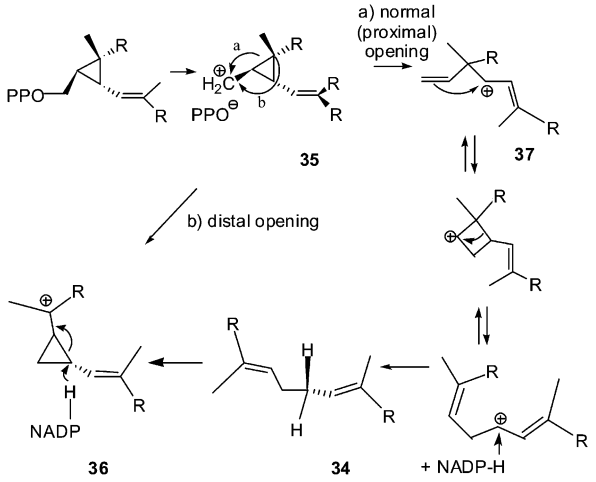
tion of a cyclopropane ring (**29**). The final skeleton results from a sigmatropic rearrangement to form the cyclopropylcarbinol (**30**, Scheme 10). The cationic cascade is terminated by water addition to yield verrucosan-2β-ol (**22**). The outlined mechanism complies with the minimal requirements for bond-making and bond-breaking processes based on experimental data, such as the formation of six carbon bonds and the disruption of two carbon bonds. Furthermore, it provides a rationale for the stereochemical course of the events which lead to the formation of the isopropyl side chain of verrucusan-2β-ol (**22**).[50]

The formation mechanisms of cyclopropanes discussed above proceed by intramolecular ring closure within an isoprenoid precursor. However, it is also known that two molecules can condense. This takes place in the case of presqualene diphosphate biosynthesis, which is the first dedicated step in cholesterol and steroid biosynthesis.[51] Presqualene diphosphate (PSPP, **31**) is formed by head-to-head condensation of two farnesyl diphosphate molecules (FPP, **18**, Scheme 11).[52−56]

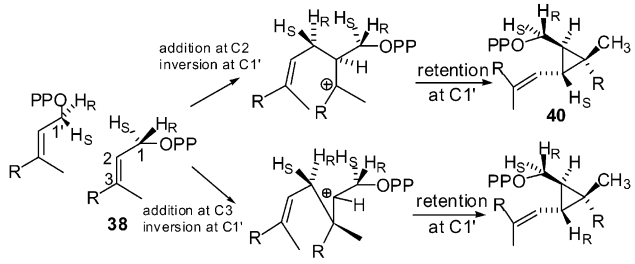
Scheme 11 indicates that presqualene diphosphate (PSPP, **31**) can be formed by electrophilic alkylation of the C(2,3) double bond of one farnesyl diphosphate (**18**) to form the protonated cyclopropane intermediate (**32**), similar to the mechanism discussed above in the casbene synthesis.[57] Alternatively, Altman et al.[58] suggested the formation of a tertiary cation as an intermediate step, with a deprotonation at the allylic β-carbon and cyclopropane formation (**33**, Scheme 12). In addition, a mechanism discussed by Trost and Biddlecom[65] for chrysanthemol formation (cf. Scheme 16) may also be applied for PSPP formation if redelivered diphosphate is used as the nucleophile instead of water.

Despite these clear but simplified mechanistic suggestions, the overall mechanism from FPP to squalene, catalyzed by squalene synthetase,[59−61] is complex and requires NADPH as a cofactor.[62] Several mechanisms with the participation of an enzyme have been discussed in the literature[52,62−67] and are referred to by Liu and Walsh.[1] Continuing from PSPP

Scheme 13



Scheme 14



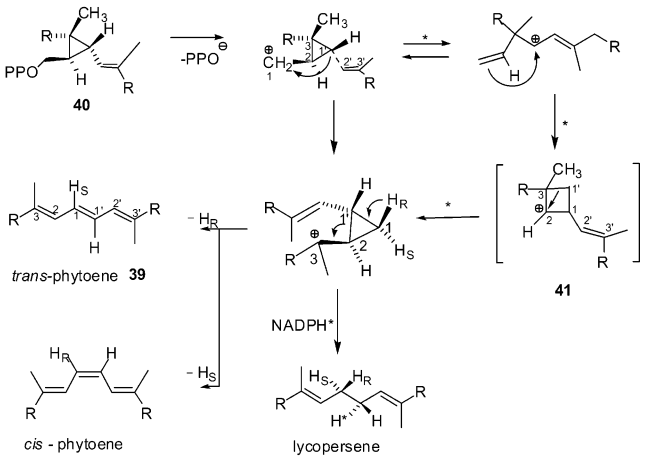
(**31**), the next step in cholesterol biosynthesis[52] is the formation of squalene (**34**) by a cyclopropane ring opening. Zhang and Poulter[60] obtained evidence for carbocationic intermediates (**35**, **119**) in this rearrangement by using recombinant yeast squalene synthethase (Scheme 13, R = $C_{11}H_{19}$ = homogeranyl).

One of the main steps in this mechanism is the hydride transfer from NADPH.[51,68] However, the "normal" opening of the cyclopropylmethyl cation would involve a proximal bond to form a homoallyl cation (**37**), instead of the distal cyclopropane σ-bond, as suggested by Zhang and Poulter.[60]

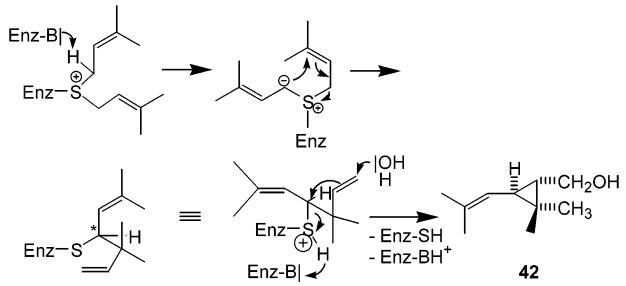
The path is also consistent with the interchange of the cationic species depicted in Scheme 3, and can be suggested for phytoene synthase, too (Scheme 15). Phytoene belongs to the group of carotenoids, naturally occurring pigments and electron donors which are formed along the novel deoxyxylose pathway (DXP pathway to isoprenoids, also called the MEP or non-mevalonate pathway).[69] The key step is the head-to-head condensation of two molecules of geranylgeranyl diphosphate (**38**, Scheme 14) to yield finally, after dehydrogenation, phytoene (**39**, Scheme 15).[70] Analogous to the biosynthetic route to squalene, the cyclopropane intermediate prephytoene diphosphate (**40**, R = $C_{16}H_{27}$ = homofarnesyl) is formed,[67] and the participation of a phytoene synthetase was proposed (Schemes 14 and 15).[71−73]

Chrysanthemol (**42**, Scheme 16) and related compounds derived from geranyl or farnesyl diphosphate are precursors of the pyrethroid-type insecticides. They show the same substitution pattern as presqualene diphosphate (cf. compound **31** in Schemes 11, 12, and 14). Accordingly, a similar cationic mechanism with a corner-protonated cyclopropane or

Scheme 15



Scheme 16



a β-carbon deprotonation can be assumed for their biogenesis. In one hypothesis, Trost and Biddlecom,[65] among others, discussed an alternative mechanism via a sigmatropic rearrangement of a sulfur ylide, followed by an S_Ni-type cyclization to form the ring (Scheme 16).

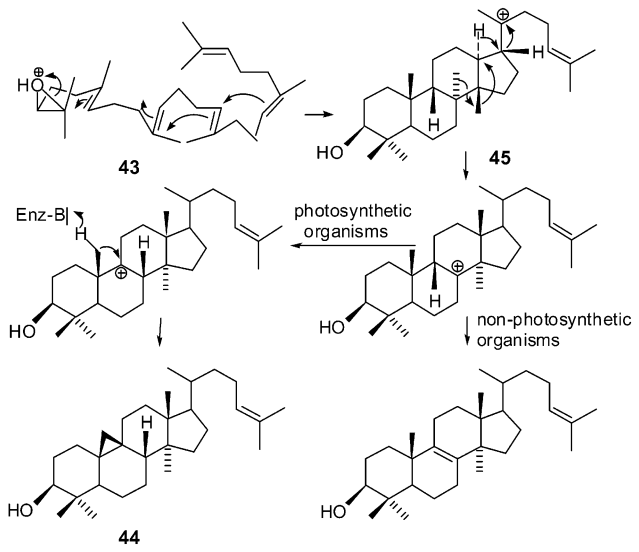
The intramolecular nucleophilic attack on the cyclopropane ring of chrysanthemol (**42**) and homochrysanthemol was studied by Herbertz and Roth by electron-transfer photochemistry in vitro, supported by quantum chemical calculations.[74,75] However, there is not clear relevance of these data for understanding the photodecomposition of chrysanthemol derivatives and for in vivo biosynthetic processes.

2. Reaction of an Intermediate Cation with an Existing α-Methyl Group

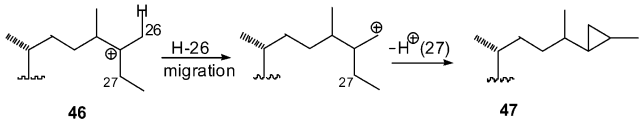
The biosynthesis of steroids has been investigated extensively.[76−79] The formation of steroids starts from squalene 2,3-epoxide (**43**, Scheme 17), which is formed via a cyclopropane intermediate as shown previously. The initial cyclization to **45** is followed by a series of methyl shifts. In photosynthetic organisms, the most important cyclization product is cycloartenol (**44**).[80,81] The cyclopropane ring is formed by incorporation of the angular methyl group under proton loss. The transformation is supported by the enzyme cycloartenol cyclase (cf. Scheme 2c).[82] However, not all details of this mechanism are known (for a discussion of possible stereochemical requirements, see Liu and Walsh[1]).

Cyclopropane rings in steroids are not only found as annealed rings; especially in nonphotosynthetic organisms, they are often formed in the side chain. Sterols with a cyclopropane ring have been isolated

Scheme 17



Scheme 18



almost exclusively from marine sources. Some of them have been known for decades, e.g., gorgosterol, 23-demethylgorgosterol, 4-methylgorgosterol, calysterol, petrosterol (**47**), or hebesterol, whereas others are relatively new, like 23-epidihydrocalysterol.[83−88] These compounds mostly stem from coelenterates,[89] soft corals,[90] or sponges.[83,83] They are usually isolated in very small amounts, and their function in nature has not yet been clarified. Endogenous bacteria living within these organisms may be the real producers.[91−93]

In analogy to the α-methyl cation ring closures, the intermediate **46**, after H^+ migration, may lead to petrosterol[94] (**47**, Scheme 18, only side chain shown), which was isolated from the sponge *Petrosia ficiformis*.[95]

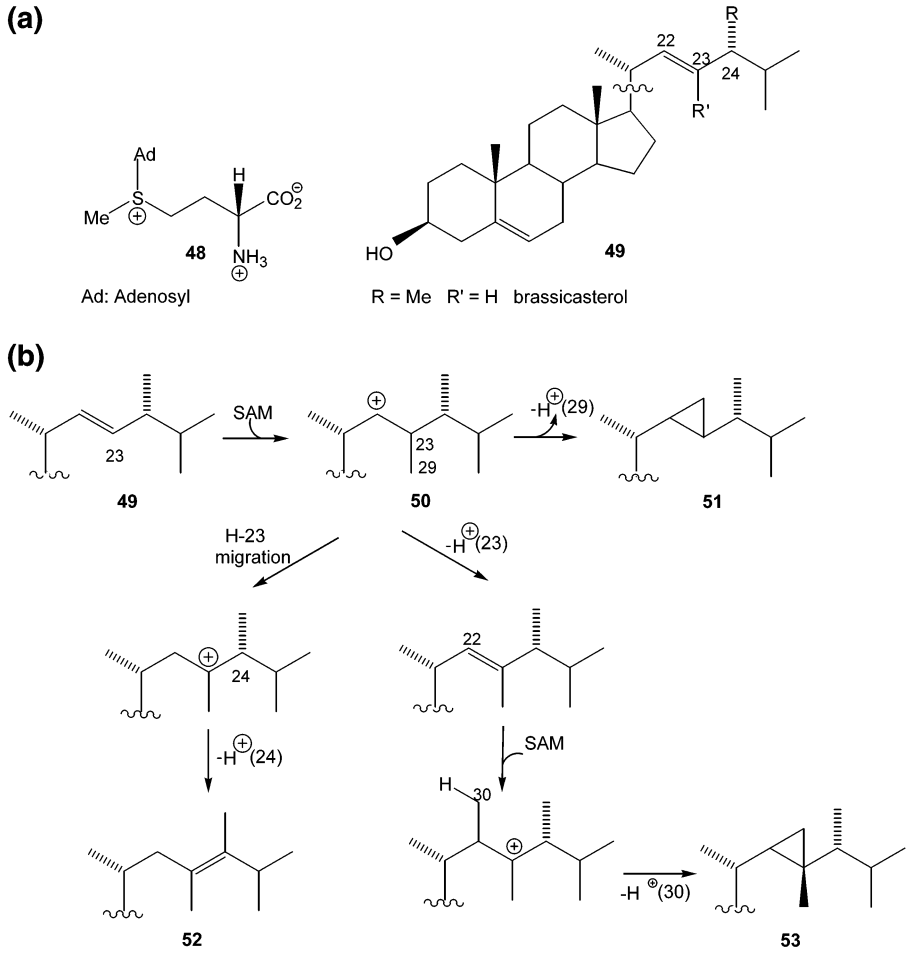
For petrosterol and the formation of Šormosterol, which was isolated from the marine sponge *Lissodendoryx topsenti*, similar mechanisms involving the participation of SAM have been discussed by Silva and Djerassi.[96]

3. Cyclopropane Ring Formation with Participation of S-Adenosylmethionine (SAM)

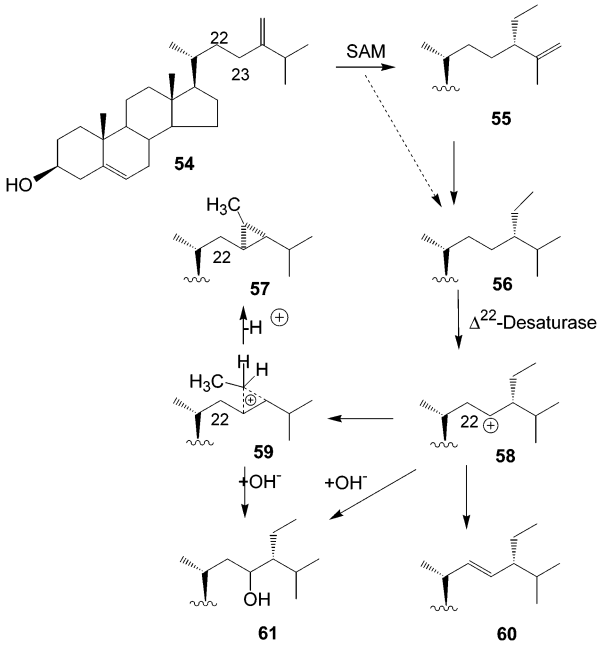
It is widely accepted that SAM (**48**, Scheme 19) plays a key role in the formation of cyclopropane rings.

Sterols. In the side chain of sterols (e.g., brassicasterol, **49**), an enzymatic methyl-transfer reaction from SAM (**48**) to a double bond is the most common pathway for formation of cyclopropane rings (Scheme 19). Starting, for instance from brassicasterol (**49**), a transferase introduces the methyl group of SAM at C(23) to give intermediate **50**, which collapses to 23-demethylgorgosterol (**51**) after loss of proton H-29. The involvement of the carbocation intermediate **50** is supported by the observation of **52** and of gorgosterol (**53**), which is formed by a second methyl transfer.

Scheme 19

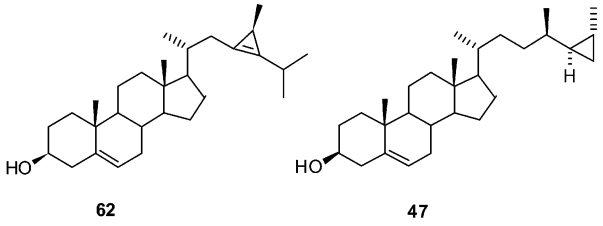


Scheme 20



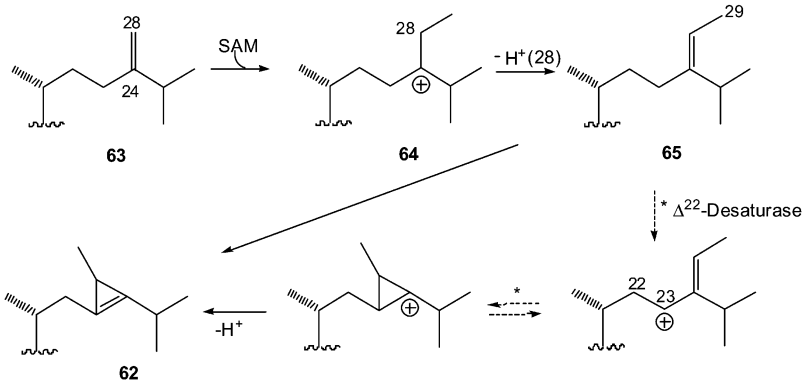
Giner et al.[97,98] suggested a slightly different mechanism for cyclopropane formation in marine sterols, involving a desaturase and a corner-protonated cyclopropane intermediate. They found that 24-methylenecholesterol (**54**, Scheme 20) was converted to clerosterol (**55**), clinasterol (**56**), and dihydrocalysterol (**57**). However, in experiments with [^{3}H]SAM in a cell-free extract of *Cribrochalina vasculum*, they found exclusive formation of clerosterol (**55**). They concluded that, from clionasterol (**56**), a deprotonation at C-23 supported by a P-450-Δ^{22}-desaturase[99] leads to the secondary carbenium ion **58** (Scheme 20). This cation triggers proton elimination at C-22 to form a double bond (**60**), adds water to yield 23-hydroxysterol **61**, or reacts to the protonated cyclopropane intermediate **59**, which can collapse to dihydrocalysterol (**57**). An analogous mechanism has been discussed for the formation of 23-epidihydrocalysterol.[83] Again, the biochemical precursor to this compound is clinasterol (**56**).

Scheme 21



The existence of a protonated cyclopropane intermediate has been demonstrated in the biosynthetic pathways to 24-propylidenecholesterol and 23,24-dihydrocalysterol by Giner and Djerassi[100] and Proudfoot and Djerassi,[101] respectively. The compounds have been found in *Chrysophyte* algae[102] and in *Calyx*

Scheme 22



niceaensis and *Petrosia ficiformis*.[103] Giner and Djerassi propose the transfer of the methyl group from SAM to isofucosterol, which forms the protonated cyclopropane.

Slightly different mechanisms have been discussed[1] for the biosynthesis of calysterol (**62**, Scheme 21), a rare cyclopropene-containing natural product, and of petrosterol (**47**). A mechanism suggested[104] for the biosynthesis of calysterol starts from 24-methylenecholesterol (**63**, only the side chain shown), to which SAM adds a methyl group in the first step (Scheme 22). β-Proton elimination of the resulting carbocation (**64**) leads to fucosterol (**65**), which then can be converted to calysterol (**62**) and two other cyclopropene isomers interconnected by double-bond migration.[105,106] We suggest a mechanistic explanation toward this end (steps marked with an asterisk in Scheme 22), utilizing the same P-450-Δ^{22}-desaturase already employed for the same transformation on the saturated side chain of clinosterol (**56**, cf. Scheme 20). This will produce an allyl cation and might be the first example of an allylic cation ring closure, i.e., the inverse of the cyclopropyl cation-to-allyl cation ring opening. The process will be driven by the enzymatically induced steric bias and the final loss of a proton.

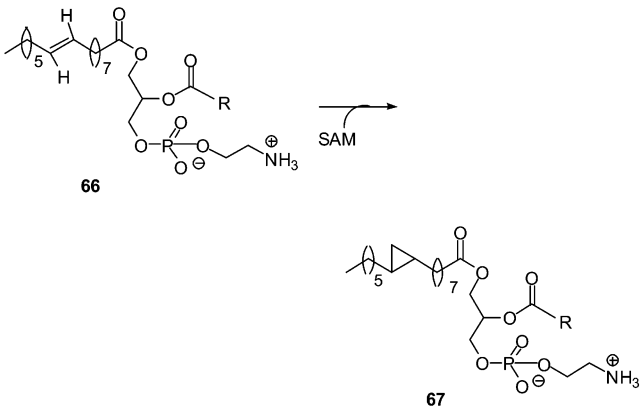
Fatty Acids. Cyclopropane-containing fatty acids are often observed in bacterial and plant lipids.[107] Cronan et al.[108] investigated the biosynthesis of cyclopropane fatty acids in *Escherichia coli*. They concluded that SAM is the methylene donor in the biosynthesis of cyclopropane fatty acids.

One of the first detailed studies with purified enzyme preparations from *Clostridium butylicium*[109] used diacyl phospholipid (**66**, Scheme 23) instead of free olefinic fatty acid as substrate. With the help of SAM, this is converted to the cyclopropane-containing fatty acid derivative **67**.

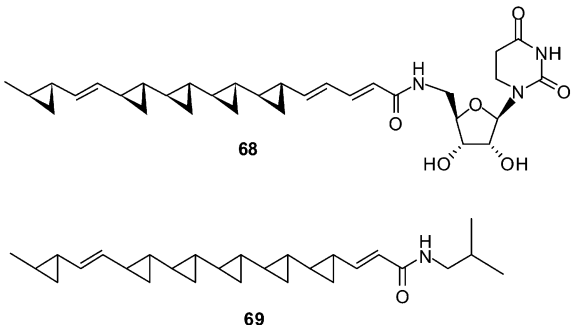
The influence of pH on the biosynthesis of cyclopropane fatty acids was studied by Sinyak et al.[110] They showed that the formation was stimulated by elevated proton levels (i.e., at lower pH) but was inhibited by increasing OH^- concentration.

Most natural compounds contain only one cyclopropane unit. However, fatty acid derivatives hold the record with respect to the number of cyclopropanes per molecule. Yoshida et al., in 1990,[111] isolated the highly biologically active fungicide FR-900848 from fermented *Streptoverticillium fervens* (Scheme 24), which contains five cyclopropane rings (**68**), as

Scheme 23



Scheme 24

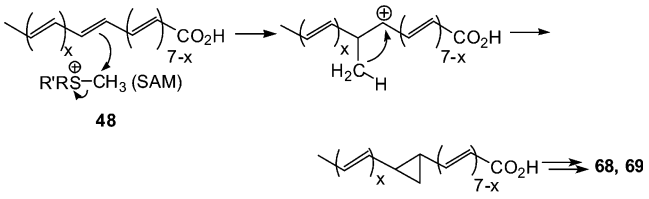


was proven by synthesis and X-ray crystallography.[112,113] A compound that carries altogether six cyclopropane rings is U-106305 (**69**, Scheme 24), isolated from *Streptomyces* species by Upjohn Laboratories.

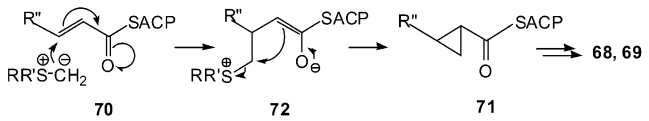
Kuo et al. showed that the C_{18} backbone of the fatty acid is formed by head-to-tail-linked acetate units common to polyketide and fatty acid pathways, whereas the cyclopropane methyl carbon stems from a methionine.[114] From these findings, the authors suggest that the polyeneoic acid of U-106305 is derived from the polyketide pathway. The cyclopropanation is achieved by Me^+ donotion from SAM (**48**) in the usual way, with subsequent ring closure and deprotonation, as shown in Scheme 25 (cf. also Scheme 2e).

Barrett et al.[115] propose an alternative mechanism, which is shown in Scheme 26 and is derived from intermediates of the normal fatty acid pathway. Cyclopropanation starts from the reaction of the

Scheme 25



Scheme 26



2-alkenoic thioester **70** with an SAM-derived sulfur ylide and proceeds to ring-closed cyclopropanecarboxylate **71** via the enolate intermediate **72** in a mechanism identical to sulfur ylide-promoted cyclopropanations in synthetic chemistry (related to the Corey−Chaykovsky reaction).

Other compound classes are also synthesized by the action of SAM on double bonds, or at least such processes are considered the most probable ones. Examples include various cyclopropane amino acids, such as hypoglycine A (**77**) or methanoproline (**91**), and spiro[2,5]octane alkaloids, such as CC-1065 (cf. **178**, Scheme 63), to name a few. Details of some of these compounds will be discussed in subsequent sections.

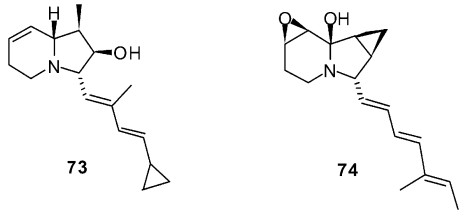
B. Internal Nucleophilic Substitution (S_Ni)—Cyclopropane Amino Acids

In 1982, the isolation and structural elucidation of an indolizidine alkaloid produced by *Streptomyces* species was reported, and this compound was called cyclizidine (**73**).[116] A similar compound (indolizomycin, **74**, Scheme 27) was subsequently isolated, also from *Streptomyces* species.[117]

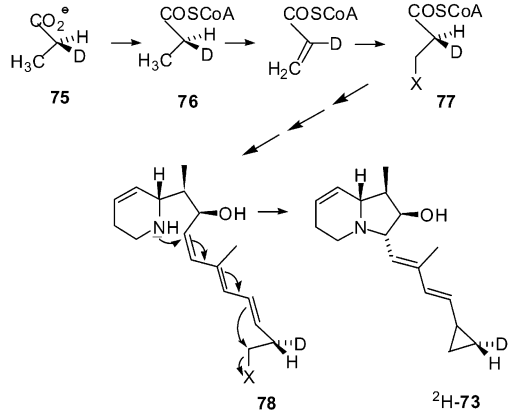
Cyclizidine (**73**) is quite unusual because it contains a terminal cyclopropyl group. The biosynthesis of cyclizidine has been extensively investigated by Leeper et al.[118] They propose the mechanism shown in Scheme 28 as the one "most consistent with all the evidence currently available".

They showed that the cyclopropane ring is derived from a propionate unit (**75**). In the first step, a propionyl-coenzyme A (CoA) complex (**76**) is formed which is utilized in the polyketide chain assembly. Michael addition of HX (X could be OH or some other functionality which can act as a leaving group) to the acrylate leads to **77**. Preceding or subsequent assembly of the rest of the molecule (cf. **78**) sets the stage for a triple vinylogous homo-S_Ni substitution. The leaving group X is involved in the formation of both the cyclopropane ring and the five-membered ring to form deuterated **73** ([^{2}H]**73**).

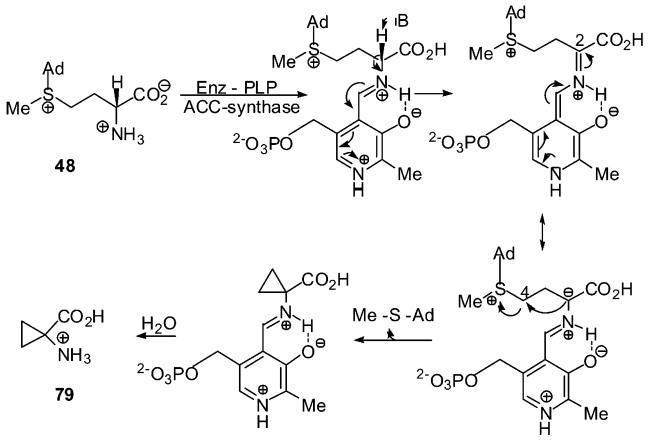
Scheme 27



Scheme 28



Scheme 29

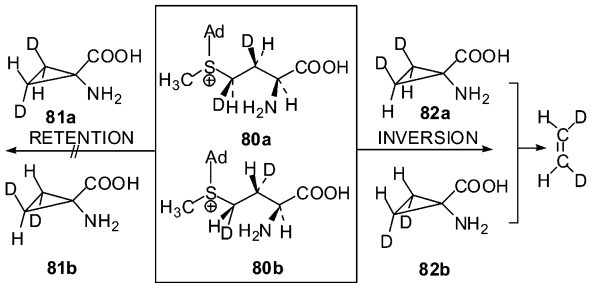


The most important class of cyclopropane-containing compounds, in which the cyclopropane ring is produced by an S_Ni mechanism, encompasses the 1-aminocyclopropane-1-carboxylic acids derived from SAM.

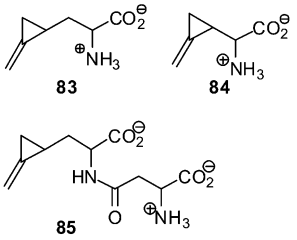
The simplest cyclopropane-containing amino acid is 1-aminocyclopropane-1-carboxylic acid itself (ACC, or ACPC, **79**, Scheme 29), which is the crucial and immediate precursor of the important plant hormone ethylene. Ethylene, among other things, is involved in senescence, fruit ripening, and interspecies communication in plants. ACC was isolated from many fruits and plant tissues.[13−15] Adams and Yang[15] detected that ethylene is formed from methionine, with ACC as the kinetically and chemically stable intermediate. In the first step, however, methionine is enzymatically converted to SAM (**48**). In contrast to the aforementioned mechanisms, the cyclopropane formation proceeds via anionic intermediates by an S_Ni reaction (cf. Scheme 2j). It was proven that ACC synthase, an enzyme first isolated from tomato,[119,120] together with pyridoxal-5′-phosphate (PLP) as a coenzyme, catalyzes the formation of ACC (Scheme 30).[121]

Wiesendanger and Boller et al.[122] investigated the stereochemical course of the biosynthesis of ACC. They showed that, under the action of a synthase from ripe tomatoes, a direct S_N2-type nucleophilic displacement of the leaving group occurs under

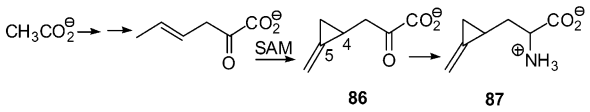
Scheme 30



Scheme 31



Scheme 32



inversion at C4. A 1:1 mixture of (3*S*,4*R*)-[3,4-$^2H_2$]- and (3*R*,4*S*)-[3,4-$^2H_2$]-(2*S*)-adenosylmethionine (**80a**,**b**) was transformed into a 1:1 mixture of the meso isomers of [$^2H_2$]-1-aminocyclopropanecarboxylic acids (**82a**,**b**) and chemically converted to [(*Z*)-$^2H_2$]ethylene (Scheme 30). The racemate **81a**,**b** was not formed.

Other types of cyclopropane amino acids may also be formed by S_Ni reaction but also by other mechanisms. Since their exact biosyntheses in most cases are not yet fully clarified, several possibilities are discussed in the following paragraphs.

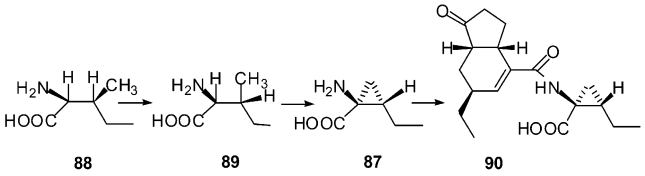
An example is the toxic amino acid hypoglycine A (**83**), which was first isolated from the akee plant (*Blighia sapida*) and has strong hypoglycemic activity.[16−18] Other related compounds, such as α-(methylenecyclopropyl)glycine (**84**) and γ-glutamyl peptides (**85**), have been found in Sapindaceae, Hippocastanaceae, and Asteraceae families (Scheme 31).[19−22]

The biosynthetic pathway to hypoglycine (**83**) has not yet been resolved completely. Several studies using labeled isoleucine or methionine led to the suggestion shown in Scheme 32.[1,123] Condensation of three acetates may give a linear C_6 acid, which is further converted to the cyclopropane-containing α-ketoacid **86** by addition of the methyl group from SAM. Unfortunately, neither a detailed mechanism for the double bond formation nor any proof of this step was presented. Transamination of **86** finally will yield hypoglycine (**87**).

A further cyclopropane amino acid with increasing biological relevance is coronamic acid (**87**).[124] Coronamic acid is formed from L-isoleucine (**88**) via L-alloisoleucine (**89**, Scheme 33).

It has been postulated that coronamic acid is synthesized by the peptide synthetase CmaA under participation of the hydrolase CmaT.[125] In this process, L-isoleucine or L-alloisoleucine is activated by adenylation and linking as a thioester to CmaA with a 4′-phosphopantetheine moiety. It is then cyclopropanated to the thioester of coronamic acid. CmaT probably hydrolyzes the thioester bond to CmaA. The amide of coronamic acid, with the bicyclic coronafacic acid, forms coronatine (**90**), which is a chlorosis-inducing phytotoxin produced by several pathovars of *Pseudomonas syringae* and acts as a potent elicitor of plant defense mechanisms.[126−129] The stereochemistry of the amino acid proved to be crucial for biological activity.

Scheme 33



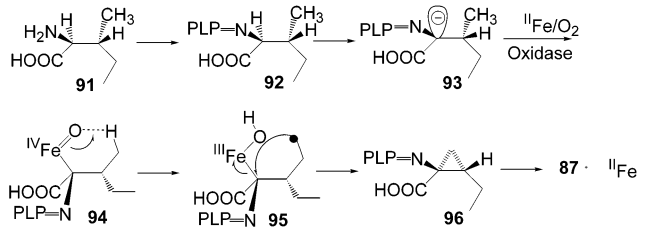
C. Transition Metal-Assisted Radical Cyclization

In addition to the above-described mechanisms, Parry et al.[130] have suggested that the synthesis of coronamic acid (**87**) is similar to that of isopenicillin-*N*, deacetoxycephalosporin-*C*, and clavaminic acid (Scheme 34).

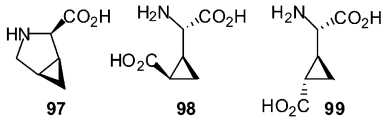
According to this, L-alloisoleucine (**91**) would react with pyridoxal phosphate (PLP) to yield the Schiff base **92**, which can be deprotonated to give the stabilized α-carbanion **93**. Reaction with the iron center of an oxidase (e.g., of the P^{450} type) and oxidation by molecular oxygen would yield the reactive Fe^{IV} intermediate **94** (cf. Scheme 2g). After abstraction of a hydrogen atom from the C-6 methyl by the iron-oxo group, the carbon radical **95** would result, which can substitute the iron by internal reaction to yield the PLP adduct of coronamic acid (**96**). Finally, hydrolysis would give free coronamic acid (**87**).

Many other cyclopropane amino acids are known. Caveney et al.[131] showed that many species of *Ephedra* produce cyclopropane amino acid analogues of proteinogenic amino acids, e.g., of glutamate and proline, in their stems, roots, and seed endosperms. One member, *cis*-3,4-methanoproline (**97**, Scheme 35), has been known for a rather long time and was

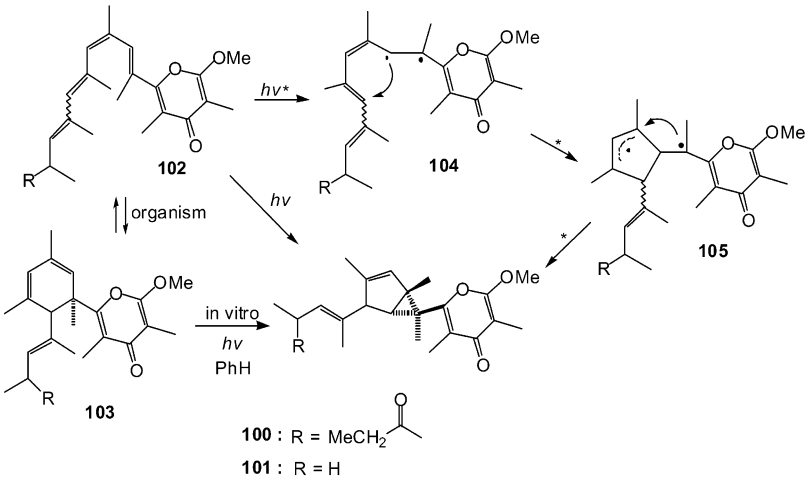
Scheme 34



Scheme 35



Scheme 36



first isolated from *Aesculus parviflora*.[132] Its X-ray structure was published by Fujimoto et al. in 1971.[133] It has been reported that *cis*-3,4-methanoproline is toxic to the bacteria *Escherichia coli* and *Salmonella typhimurium* through its feedback inhibiton of proline synthesis.[134]

Two other cyclopropane-containing amino acids, *cis*-α-(carboxycyclopropyl)glycine (**98**) and its trans analogue **99**, have been isolated from *Aesculus parviflora* and *Blighia sapida*[132] but occur also in several *Ephedra* species.[131]

These compounds are known to be potent blockers of high-affinity Na^+-dependent glutamate transport in the mammalian central nervous system and in insect tissues.[135−137] However, nothing is known yet about their metabolism, although it can be suspected that they are produced in a manner similar to the production of coronamic acid (SAM-mediated or Fe-catalyzed).

D. Photoinduced Cyclopropane Formation

Crispatene (**100**) and erispatene (**101**, Scheme 36) have been isolated from *Tridachia crispate*, a mollusk species assimilating chloroplasts from siphonous algae.[138] It is very likely that these compounds are formed through a photochemical cyclization mechanism. In this case, the hexatriene **102** might well be the precursor of crispatene (**100**) and its analogues (**101**, Scheme 36).[139] This assumption was supported by in vitro photolysis of 9,10-deoxytridachione (**103**, R = H), which is converted to **100**.

It was suggested that the photoreaction from **103** to **100** proceeds via a $[_{\sigma}2_a + {}_{\sigma}2_a]$ mechanism.[1,139] However, we herein suggest that either a di-π-methane rearrangement from **103** after a double-bond migration or—most likely—direct photocyclization of the triene moiety (**102**), as depicted in Scheme 36 along the path marked with an asterisk, offers an alternative pathway. This approach allows the initial excitation of the enone system to be directly transferred to the triene moiety. All intermediate radicals **104** and **105** are stabilized, and the product is formed without much constraint following the NEER (non-equilibrium of excited rotamers) principle.[140] The photoreaction of **103** to **100**/**101** in vitro can be explained by (photochemically or thermally induced) retro reaction of **103** to **102** and then triene cyclization to **100**/**101**.

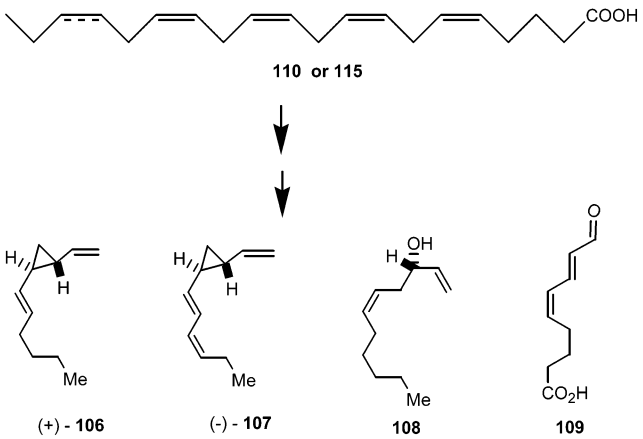
E. Other Mechanisms

The biosynthetic pathways and reactions to form cyclopropane moieties which cannot be classified clearly under any of the aforementioned mechanisms are summarized in this section.

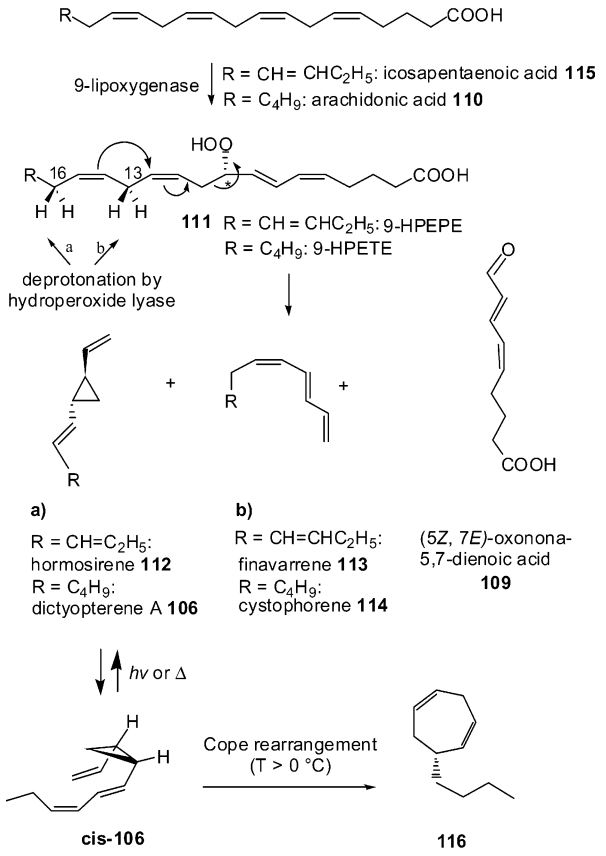
Dictyopterenes A and B (**106**, **107**) are cyclopropane-containing alkenes which are very likely metabolites (other cf. **108**, **109**) from the fragmentation of the hydroperoxide of arachidonic acid (**110**, Scheme 37).

These compounds have been isolated from seaweeds,[141] where they act as a sperm attractant pheromone for gametes.[142] Furthermore, the dictyopterenes were isolated from heterocontophytic diatoms and higher plants, where their biological purpose is unknown.[143] Hombeck et al.[144] have shown that (9*S*)-hydroperoxyicosatetraenoic acid (9*S*-HPETE, **111**, Scheme 38) is an intermediate in the biosynthesis of dictyopterene A (**106**) and also hormosirene (**112**). They demonstrated the incorporation of a single oxygen atom of labeled $^{18}O_2$ in the C9 position in arachidonic acid and icosapentanoic acid, catalyzed by 9-lipoxygenase, which leads to the initial functionalization of the fatty acid in forming 9-HPETE

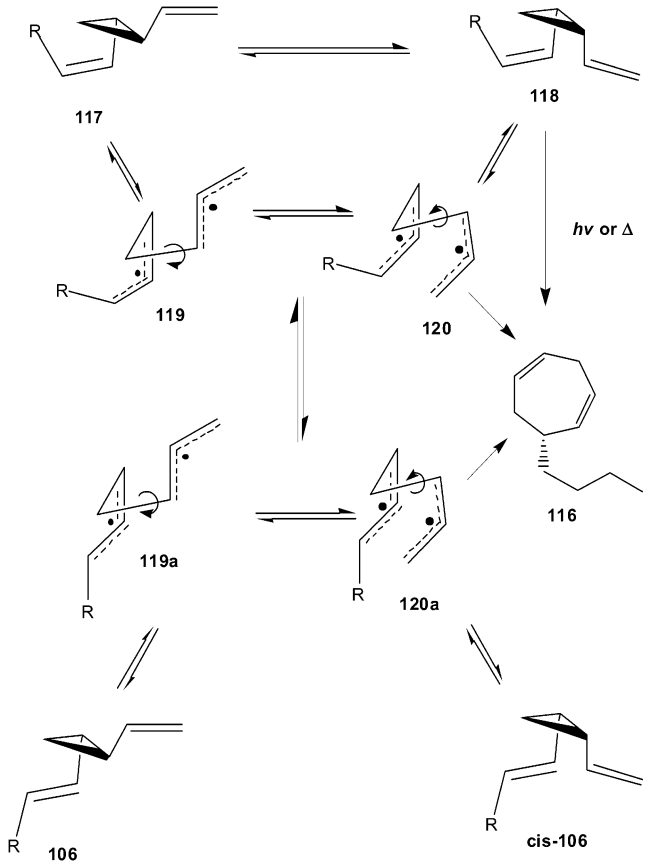
Scheme 37



Scheme 38



Scheme 39

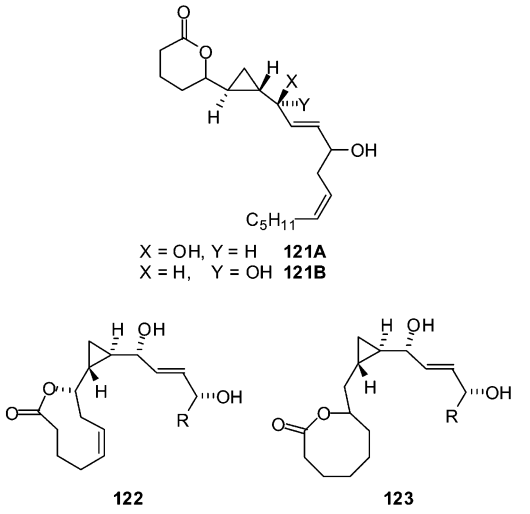


and 9-HPEPE. Internal oxidative cleavage catalyzed by a hydroperoxide lyase leads to the fragmentation products **106**−**109**, **112**−**114**, and **116** along with formation of dictyopterene A (**106**) and hormosirene (**112**). However, until now, it could not be proven whether the transformation proceeds via an ionic or a radical mechanism.

A similar mechanism, discussed by Pohnert and Boland,[145] includes a proposed Cope rearrangement of the thermally labile cis derivative to ectocarpene (**116**, Scheme 38). Since the cis compound is the biologically more relevant attractant, its thermal decomposition is crucial in order to establish a suitable concentration gradient, rather than flooding the environment with the signal. Boland and Mertes[146] showed that the diatom *Gomphonema parvulum* produces **106** via a lipoxygenase/hydroxyperoxide lyase. In this mechanism, the oxidation of arachidonic acid (**110**) is followed by enzyme-catalyzed cleavage to form **106** or **112** with lipoxygenase and hydroxyperoxide lyase, respectively, and 10-oxonona-(5*Z*,7*E*)-dienoic acid (**109**). The results of investigations of the thermal and photochemical rearrangements of divinylcyclopropanes by Pickenhagen et al. strongly support a trans−cis isomerization of **106** via biradical transition states (**119**, **120**, Scheme 39) as the primary step before the Cope rearrangement to form **116**.[147]

Other compounds which originate from arachidonic acid are the constanolactones A (**121A**) and B (**121B**) and related compounds, halicholactone (**122**), and solandelactone (**123**). These eicosanoid cyclopropane oxylipans were isolated from the red algae *Con-*

Scheme 40

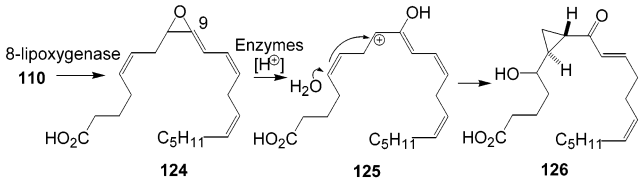


statinea simplex,[148] the corals *Plexaura homomalla*[149] and *Halichondria okadai*,[150,151] and *Solanderia secunda*,[52,152] respectively (Scheme 40).

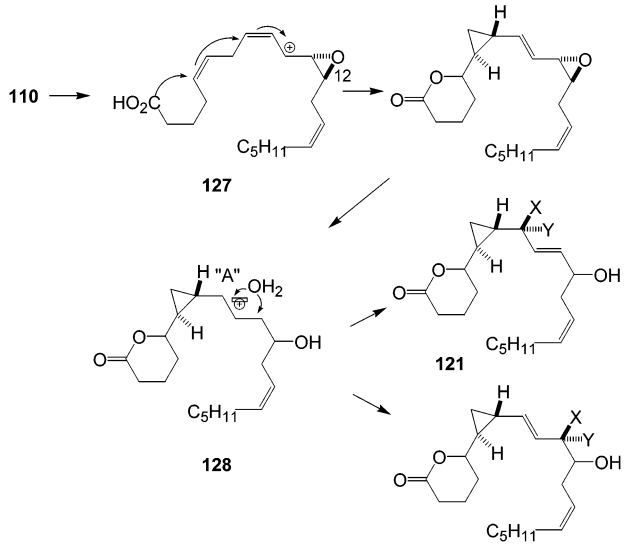
It was hypothesized that oxidation of **110** by lipoxygenase leads to the allene oxide **124**, which then can be converted to a cation intermediate **125** by opening of the epoxide.[148] Subsequent ring closure of the homoallylic cation **125**, supported by nucleophilic attack of water on the developing rearranged cation, produces the cyclopropyl compound **126** (Scheme 41).

The existence of an allene oxide intermediate has also been postulated in the formation of a cyclopropane intermediate for the biosynthesis of 5,6-*trans*-

Scheme 41



Scheme 42



prostaglandin A_2, which was isolated from the soft coral *Plexaura homomalla*.[149]

According to this mechanism, Nagle and Gerwick[148,153,154] propose that the constanolactones (**121**) and their isomers arise from the cyclization of the epoxy cation intermediate **127**, followed by nucleophilic attack of water on the allylic cation **128** (Scheme 42). A similar pathway, with carboxylate attack at the next double bond, can lead to halicholactone (**122**).

III. Degradation and Metabolism of Cyclopropane Rings in Natural Compounds

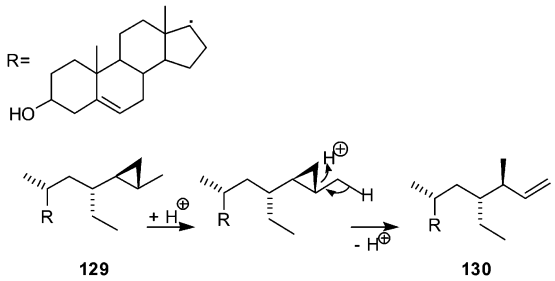
The metabolization of cyclopropane rings in natural products is much less studied than their biosynthesis. The functionalization of intact cyclopropane units in natural products is almost unknown; mostly ring-opening reactions have been reported. In contrast to the formation of cyclopropanes, their opening reactions normally are thermodynamically highly favorable and thus proceed rapidly and irreversibly if the kinetic barrier for ring opening is overcome, e.g., by acid catalysis. Specific studies of such opening reactions often concern the biological targets of cyclopropanoid compounds; e.g., their function as natural enzyme inhibitors is studied, rather than "normal" catabolism. From a mechanistic perspective, the ring-opening reactions can be considered as the reverse processes of the corresponding biosyntheses and may be grouped accordingly, as summarized in Scheme 2. However, a slightly different organization is followed here. Some transient ring-opening reactions, e.g., those of presqualene (**31**) are prephytoene (**40**), have already been discussed in the previous sections and are not repeated here. The reactions and metabolization of non-natural cyclopropanoid compounds such as mechanistic probes, inhibitors, and drugs also are not part of this review and are covered only if related to a "native" mechanism.

A. Acid-Catalyzed Cyclopropane Ring Opening by Forming an Angular Methyl Group

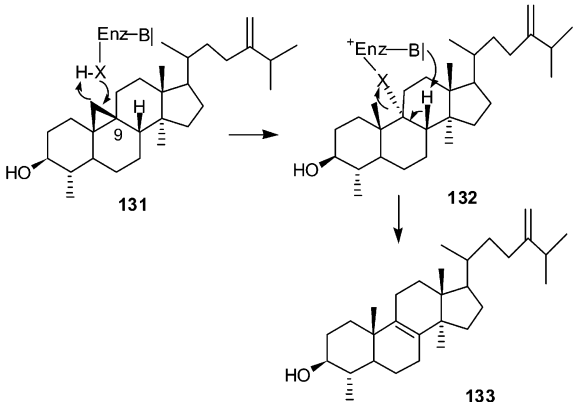
The biosynthesis of several cyclopropane-containing sterols has been discussed in section II. However, the cyclopropane group in these sterols may not be the final product in all cases. Cho and Djerassi[83] postulated that the sterol ficisterol (**130**) is a metabolic product which results from cationic cyclopropane ring opening from hebesterol (**129**, Scheme 43). They suggest that the cyclopropane ring is opened by acid catalysis and subsequently forms the double bond by β-proton loss at the terminus.

In section II, the formation of the cyclopropane ring in cycloartenol **44**, a major sterol precursor in higher plants and fungi, was discussed.[81] The cyclopropane ring of this compound can be opened under participation of an enzyme found in tissue cultures, e.g., of bramble (*Rubus fruticosus*).[155−157] The same ring opening has been investigated for the cycloartenol-related steroid cycloeucalenol (**131**, Scheme 44).[156,157] It is opened at the cyclopropane to give the enzyme-bound intermediate **132**, with an angular methyl group. Subsequently, the 8β-hydrogen is eliminated, together with the enzyme, which leads to obtusifoliol (**133**).[155] Overall, this is mechanistically exactly the reverse process of the biosynthetic cyclopropane ring formation described in Scheme 2c.

Scheme 43



Scheme 44



B. Ring Opening of Cyclopropylamines, Cyclopropanols, and Related Compounds

1. Enzymatic Decomposition of Cyclopropylamines

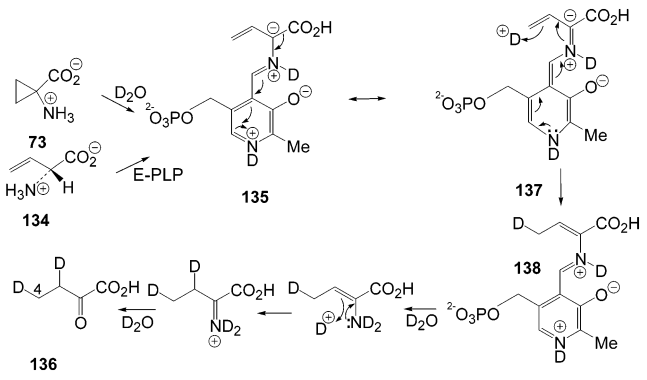
One of the most important and well-studied processes involving cyclopropanoid natural products is the enzyme-catalyzed fragmentation of 1-aminocyclopropanecarboxylate (ACC, **73**) to the plant hormone ethylene.[158,159] In bacteria and yeast, ACC is converted to α-ketobutyrate and ammonia.[159,160]

The latter process is catalyzed by ACC deaminase, which needs, in analogy to ACC synthetase, PLP as a coenzyme.[160,161] In the first step, PLP binds to the amino function of ACC (**73**). This is accompanied by cyclopropane ring opening (**135**, Scheme 45).

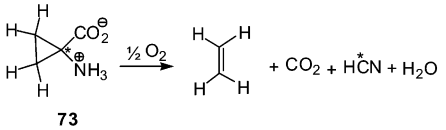
From deuterium isotope investigations,[161] it is known that one deuterium is incorporated at C3 and another at C4 of ACC to form 3,4-bisdeuterated α-ketobutyrate (**136**). From these studies, it was deduced that vinylglycyl-PLP aldimin isomers (**137**, **138**) are the key intermediates in this process. For a more detailed discussion of the mechanism, see Liu and Walsh.[1]

In plants and fruits, ACC (**73**) is oxydized to ethylene, carbon dioxide, and hydrogen cyanide in a fascinating fragmentation process (Scheme 46).[162,163] Pirrung and McGeejam[164,165] have proposed a generally accepted radical mechanism (cf. Scheme 2f) for the decomposition of the cyclopropane ring in ACC. The first step is the oxidation of the amino group by the removal of one electron, to form the cyclopropylaminium radical **139** (Scheme 47). This radical cation is unstable and decomposes to the carbon-centered primary radical and an iminium ion (**140**). The imine formed after proton loss can provide another electron to the enzyme, to yield the diradical cation **141**, which collapses to ethylene and formic acid cyanide. The latter decomposes to hydrogen cyanide and carbon dioxide. Similar processes have been studied with substituted ACC, mostly in vitro, yielding the corresponding alkenes.[162,163]

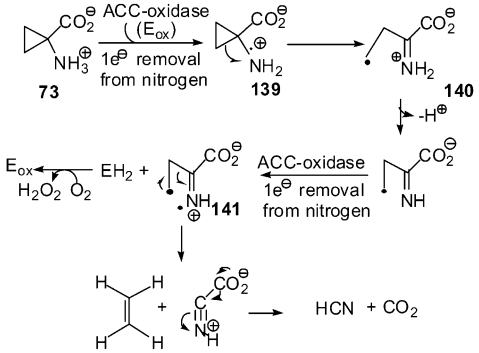
Scheme 45



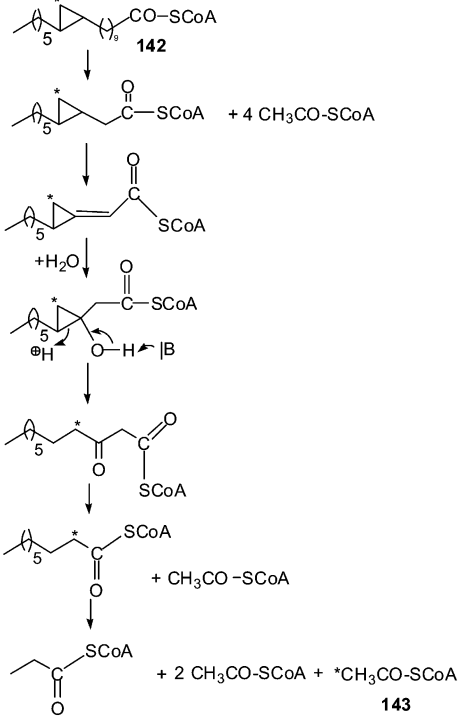
Scheme 46



Scheme 47



Scheme 48



2. Catabolism of Cyclopropane Fatty Acids

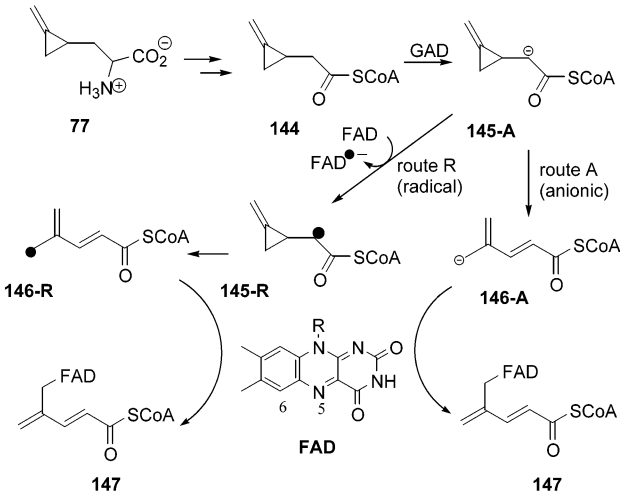
Tipton and Al-Shathir investigated the degradation of cyclopropane fatty acids in whole cells of *Tetrahymena pyriformis*.[166] From labeling experiments using *cis*-11,12-[methylene-^{14}C]methyleneoctadecanoic acid (**142**), they derived the degradation route shown in Scheme 48, which is supported by the detection of formation of [2-^{14}C]acetate (**143**).

3. Radical Intermediates in Cyclopropane Ring Opening and Enzyme Inhibition

There are many examples known where inactivation of an enzyme is related to the reactivity of the cyclopropyl group itself. Numerous synthetic cyclopropane derivatives have been designed and tested for their reactivity with enzymes as mechanistic probes or in order to explore their potential as drugs. In the following, we will focus on known radical-based mechanisms of the inactivation of enzymes involving ring opening of the cyclopropane group in natural inhibitors or closely related compounds, used to elucidate the mechanism.

Transamination and decarboxylation of hypoglycine A (**77**) give the active metabolite **144** as a

Scheme 49

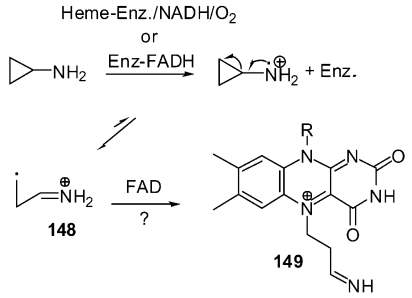


coenzyme-A-bound thioester (Scheme 49).[167] This compound is a suicide substrate for acyl-CoA-dehydrogenase (GAD = general Acyl-SCoA-dehydrogenase), a flavin adenine dinucleotide (FAD)-dependent enzyme crucial in fatty acid degradation and thus finally ATP production. The inactivation is achieved by covalent modification of the FAD-coenzyme in the active site.[1,168] Two mechanisms, one anionic (route A) and one radical (route R), have been discussed. It is hypothesized that the methylene cyclopropyl acyl-CoA is converted to an α-anion (**145-A**) when it is docked into the active site of GAD.[168] This anion may undergo cyclopropane ring opening to form the allylic, cross-conjugated δ-anion of an α,β-unsaturated ester (**146-A**)—a 1,3-dipole equivalent—which is supposed to attack first as a nucleophile at C6 of FAD to give **147**, and in addition possibly as an electrophile at N5 of FAD. However, in an alternative mechanism (route R), one electron transfer from FAD forms the analogous radical intermediate **145-R**. Ring opening of this radical should be faster than that of the anion, and the resulting radical **146-R** would react with the reduced FAD to give the identical covalent product **147** as in the anionic mechanism. Liu and co-workers have presented excellent evidence that, indeed, the radical route is most likely followed.[113]

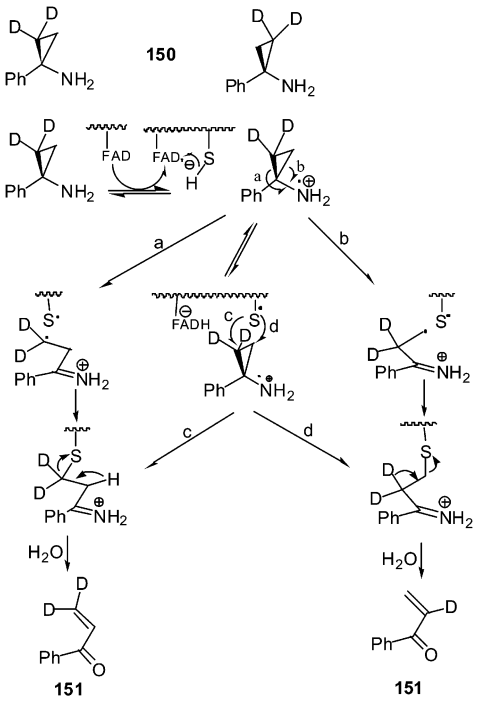
Selective inhibition of the mitochondrial flavoenzyme monoamine oxidase (MAO) was considered for the development of tranquilizing drugs.[169,170] Tranylcypramine[171] and analogous cyclopropylamines were found to be suicide inhibitors of MAO.[172−174] It has been suggested by several authors that the enzyme-bound flavin coenzyme semiquinone may oxidize the amine substrate by one-electron transfer.[172−174] As an intermediate, an aminium radical **148** is formed, which readily opens to the 3-propyliminium radical **148** (Scheme 50). The radical finally can react within the active site, e.g., with flavin, to form a covalent adduct such as **149**. A two-electron oxidation has also been discussed in the inactivation process of MAO but is considered less likely.[173,175]

Other oxidizing enzymes which can be inactivated by cyclopropylamine substrates via a one-electron-transfer mechanism are the cytochrome P_{450}-type heme enzymes.[176,177] Under participation of oxygen

Scheme 50



Scheme 51



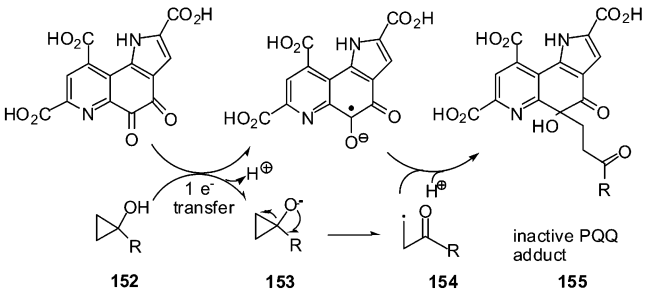
and NADPH, one electron is transferred from the nitrogen of the cyclopropylamine to the enzyme (Scheme 50). This again leads to the intermediate cyclopropylaminium radical, which is stabilized by ring opening and subsequent covalent coupling to the enzyme.

Silverman et al. investigated the stereoselective ring opening of 1-phenylcyclopropylamine (**150**) catalyzed by MAO and also proposed a radical mechanism.[178] He also showed that not only is flavin attacked irreversibly, but, alternatively, also a cysteine located in the active site is covalently bound (Scheme 51). In the latter case, the enzyme can slowly recover by the release of acrylophenone **151**. It can be concluded that, although it is somewhat thwarted by primary and secondary isotope effects, path a is followed for this process.

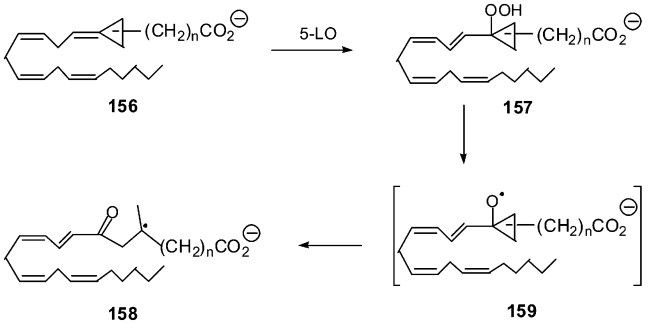
A third group of enzymes which can be modified by cyclopropane-containing substrates are certain bacterial alcohol dehydrogenases, with pyrroloquinoline quinone (PQQ) as coenzyme.[179] It has been reported that cyclopropanols (**152**) inhibit such an enzyme by an initial one-electron transfer from the oxygen atom of cyclopropanol to PQQ (Scheme 52).[180,181]

The thus-formed oxygen-centered cyclopropanol radical (**153**) decomposes to the propionaldehyd $C_β$-

Scheme 52



Scheme 53



radical (**154**), which subsequently inactivates PQQ by covalent binding (**155**) in a mechanism similar to MAO−FAD inhibition.

The enzymatic fragmentation of the hydroperoxide of arachidonic acid for the production of cyclopropanoids has been discussed in section II.E. However, hydroperoxides of cyclopropane fatty acids, namely cyclopropylidene intermediates (cf. Scheme 48), may also contribute to ring-opening and inhibition reactions of enzymes from the arachidonic acid-based pathways. Leukotrienes are lipoxygenase-derived products of the arachidonic acid metabolic pathway. Substrate-modeled inhibitors of the leukotriene biosynthesis for the inhibiton of $^5\Delta$-lipoxygenase (5-LOX), e.g., methylene cyclopropanes **156** (Scheme 53), have been developed by Misra.[182]

He proposes that the conversion of the hydroperoxide **157** by homolytic cleavage will lead to **159** and subsequent cyclopropyl ring opening. The resulting β-keto radical **158** can inactivate the enzyme by binding to a nucleophilic position in the active site.

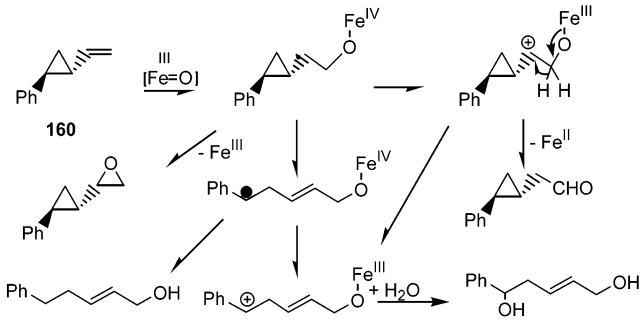
C. Transition Metal-Assisted Cyclopropane Ring Opening

A mechanism similar to the P_{450} oxidation of cyclopropylamines (cf. Scheme 50) has been proposed by Miller et al., using *trans*-1-phenyl-2-vinylcyclopropane (**160**, Scheme 54) as a hypersensitive radical probe for oxidation by cytochrome P_{450} (cf. also Scheme 2g).[183]

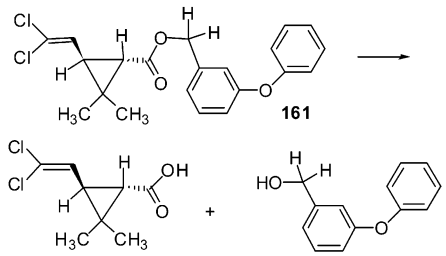
D. Metabolism at the Cyclopropane Ring or at Its α-Position without Opening

Pyrethrins and pyrethroids (e.g., **161**) are known as potent insecticides.[2] Several studies have investigated the metabolism of this class of compounds.[184−187] However, most of the studies did not consider opening of the cyclopropane ring but found

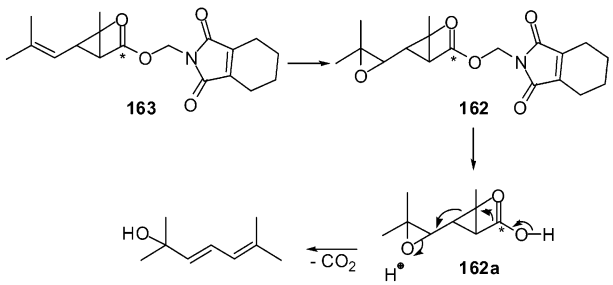
Scheme 54



Scheme 55



Scheme 56



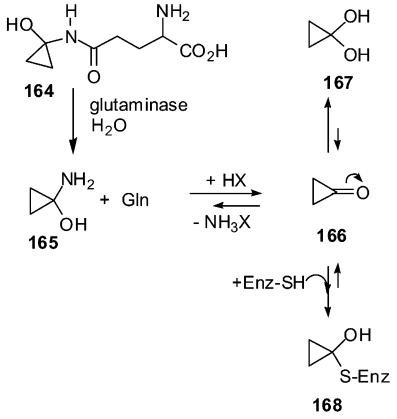
only products resulting from ester hydrolysis (e.g., Scheme 55).

Smith and Casida showed that epoxychrysanthemic acid (**162**) is an intermediate of the chrysanthemate insecticide **163**, which gives ring opening under decarboxylation (Scheme 56).[188]

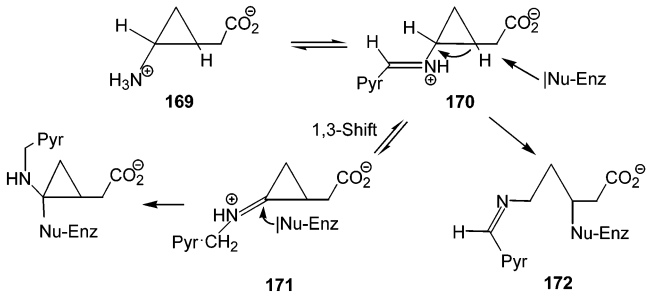
Coprin (**164**) is a γ-glutamylamide cyclopropanone hemiacetal isolated from the mushroom *Coprinus atramentarius*. It is stable in aqueous solution and causes severe effects when used together with alcohol. In mammals (or in bacteria), it can be hydrolyzed to liberate cyclopropanone hemiaminal (**165**), which is in equilibrium with cyclopropanone (**166**) and possibly the corresponding hemiacetal (**167**, Scheme 57). Cyclopropanone is extremely reactive with all nucleophiles, because addition to the cyclopropanone sp^2-carbon releases some of the ring strain while forming the sp^3-hemiacteal. It also has minimal steric hindrance (comparable to, e.g., that of acetaldehyde). Within the active site of aldehyde dehydrogenase, cyclopropanone is able to react with the cysteinyl thiolate side chain of the enzyme and forms a stable covalent hemithioacetal−enzyme complex (**168**), which leads to the loss of activity.[189]

Cyclopropane analogues of γ-aminobutyric acid have been synthesized as active-site-directed, mechanism-based inhibitors of GABA-transaminase and a homogeneous bacterial ω-amino acid: pyruvate transaminase.[190] According to Scheme 58, cyclopro-

Scheme 57



Scheme 58

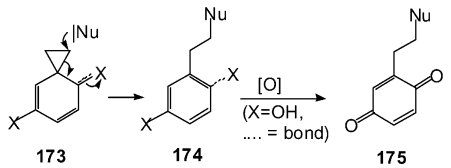


pylamino acid **169** may be activated in the enzyme as aldimine **170**, which allows an equilibrium with the somewhat less favorable **171** by a 1,3-proton shift. The cyclopropyliminium ion **171** should readily react with nucleophiles, e.g., from amino acid side chains in the active site, as reported above for the formation of cyclopropanone from coprine. Another inhibition pathway may be the direct opening of the activated **170** by a homo-S_N2' reaction, to give **172**.

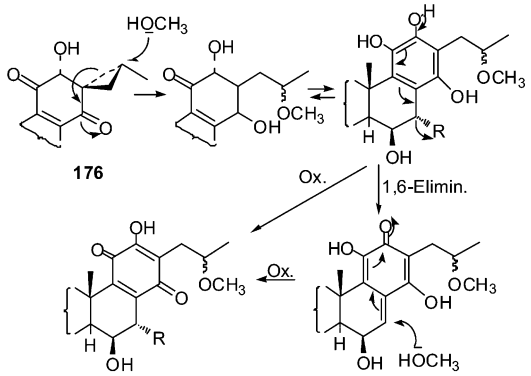
E. Nucleophilic Opening of Activated Cyclopropane Trigger Compounds

Another general mechanism of ring opening is the nucleophilic attack at a cyclopropane connected to an acceptor group or an α-leaving group to give a homo-Michael−Addition or a homo-S_N2' substitution, respectively, strongly driven by the liberation of the ring strain energy (Scheme 59). A common structural element that achieves this are spiro[2,5]-4,6-octadienes **173**, and related systems. In these compounds, a reaction cascade is triggered by nucleophile addition to the cyclopropane. The already considerable driving force and irreversibility of the ring opening is enhanced by its connection to an aromatization process (cf. **174**). Even this process can be enhanced by further (nonenzymatic) reaction of the aromatic system, usually a hydroquinone-type structure, which is easily oxidized to a quinone form (cf. **175**). In

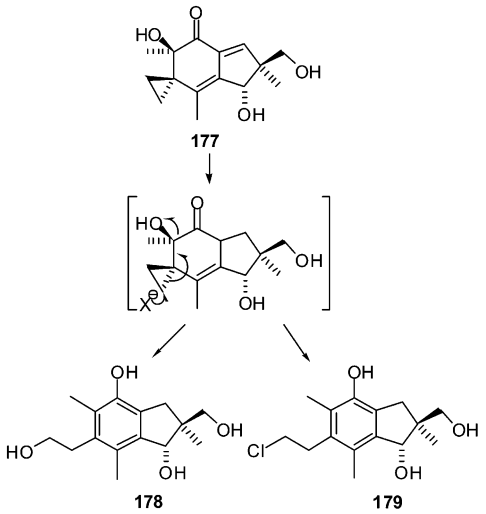
Scheme 59



Scheme 60



Scheme 61



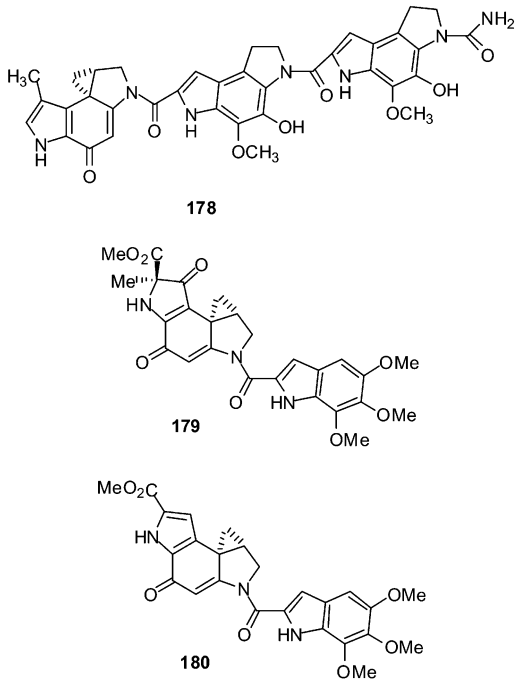
nature, the triggering may be controlled by pH, proximity effects upon binding, or protected precursors such as glycosides. Some examples of such nucleophilic ring openings are given below. However, the principal mechanism is not limited to the well-studied and fancy spiro[2,5]octadiene-type systems. Thus, the opening of chrysanthemum epoxide **162a** (Scheme 56) is an acyclic axample, and virtually any sufficiently activated acceptor-substituted cyclopropane, e.g., the curacins mentioned at the very end of this section, can potentially react by this mechanism.

Rüedi et al. investigated the methanolysis of the spiro(methylcyclopropane) moiety in four diastereoisomeric lanugons J (**176**), which were isolated from the *Coleus barbatus* group (Scheme 60 shows the reaction of one stereoisomer).[191,192] They showed that the ring opening proceeds stereospecifically by nucleophilic attack of the solvent (here methanol) with inversion of the configuration. In natural systems, nucleophiles such as thiol groups from enzymes or amino groups from DNA may act as the nucleophile.

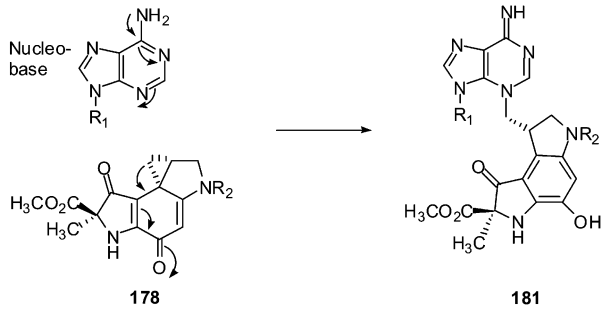
Illudin S (**177**) is a sesquiterpenoid that has been isolated from the mushroom *Lampteromyces japonicus*. Tanaka et al. proposed that ring opening of the spiro[2,5]octene moiety occurs by a homo-S_N2' mechanism (Scheme 61). Accordingly, after oral administration of illudin S to rats, products **178** and **179** have been found in urinary excretions.[45,193−196]

CC-1065 (**178**, Scheme 62) is a highly potent antibiotic exhibiting cytotoxic and antitumor

Scheme 62



Scheme 63

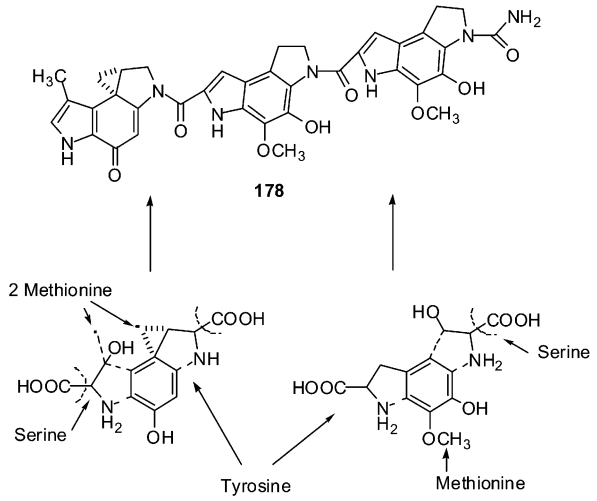


activity.[197−199] CC-1065 was first isolated from *Streptomyces zelensis* at Upjohn in 1978.[200,201] Later, it was shown to be identical to rachelmycin, which was isolated from *Streptomyces* C-329 at Bristol-Meyers[202] and also from *Streptomyces canulus*.[203] More recently, two new compounds of this class were identified in *Streptomyces* DO-88, DO-89 and DO-113, and these are called duocarmycins A (**179**) and SA (**180**, Scheme 62).

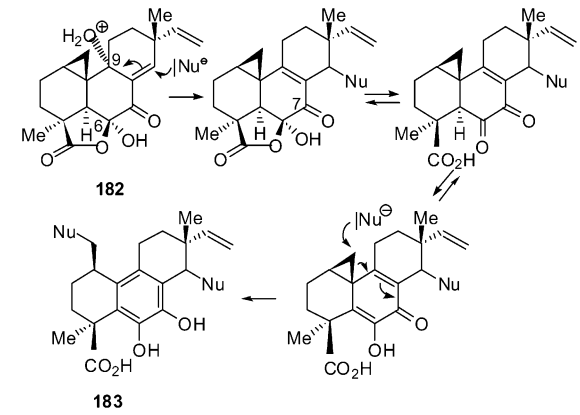
It has been shown that CC-1065 (**178**) reversibly binds to AT-rich minor groove sites of duplex DNA in a stereoelectronically controlled complex formation.[26,46,58,61,204] Extensive investigations of the mechanisms of binding of CC-1065 and duocarmycine have been carried out by Boger and Johnson.[205] Apart from possible other covalent interactions, these compounds are spiro[2,5]octadienones and can react with DNA (e.g., **181**) via nucleophilic cyclopropane ring opening (Scheme 63).

The biosynthetic pathway to CC-1065 has been investigated by Hurley and Rokem by means of radioactive isotope techniques.[206] They proposed that tyrosine is the precursor of all three benzodipyrrole subunits, whereas the methylene group needed to form the cyclopropane ring is probably transferred from SAM (Scheme 64). Various studies on the synthesis of CC-1065 and duocarmycins were described, e.g., by Boger et al.,[207] and will be discussed in our related publication.[12]

Scheme 64



Scheme 65

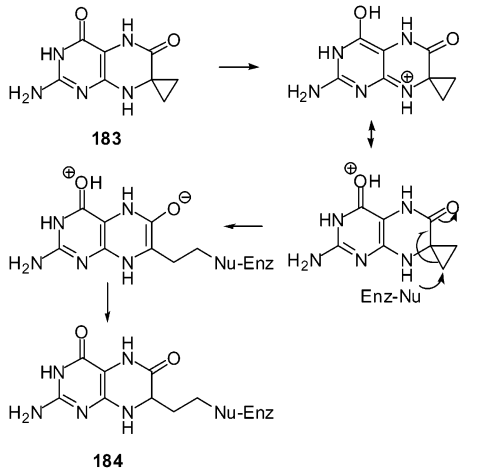


Myrocin C (**182**, Scheme 65) is a diterpene antitumor antibiotic from *Myrothecium verrucaria*[208,209] containing a cyclopropane ring in a spiro[2,5]octane unit which bears a formal relationship to the CC-1065 family of antitumor agents.[7,8,10] Chu-Moyer and Danishefsky showed that a double activation is required for the ring opening of **182** by the attack of two nucleophiles, e.g., thiols (Scheme 65).[210]

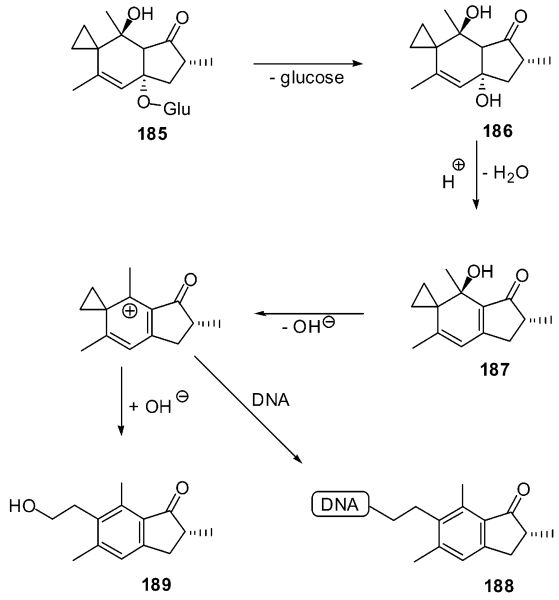
The mechanism-based inhibitor **183** was designed for dihydrofolate reductase (DHFR), carrying a cyclopropane ring as the reactive group in a spiro[2,5]-octane moiety.[211] The nucleophilic attack of an enzyme nucleophile on a methylene carbon of the cyclopropane ring opens the ring and leads to irreversible inhibition of the active site of the protein (**184**, Scheme 66). A similar inhibitor and mechanism was proposed earlier by Haddow et al.[212]

Ptaquiloside (**185**) is an unstable norsesquiterpene glucoside which has been isolated from bracken fern (*Pteridium aquilinum*) by Japanese and Dutch groups.[3,213−215] It has been shown that ptaquiloside is the active principle[213−215] that induces bladder and intestinal carcinomas when cattle consume bracken.[3,4,216] The toxic syndromes induced by this compound differ in different species and cause anorexia, bright blindness, leucopenia, thromocytopneia, and other complications.[217−220] After cleavage

Scheme 66



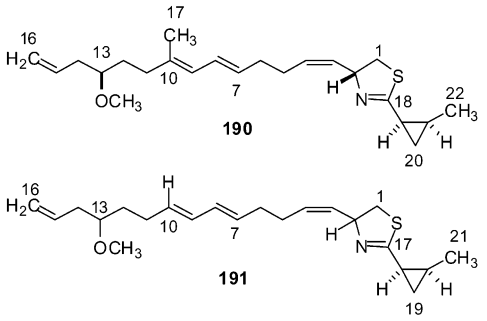
Scheme 67



of the glucoside, the reactive aglycon ptaquilosin (**186**) eliminates water, even under weak alkaline conditions, to form the reactive spiro[2,5]octadienone **187** (Scheme 67). It was shown that ptaquilosin alkylates adenines and guanines in DNA in a sequence-selective fashion (cf. **188**) or hydrolyzes to pterosin (**189**).[221] Recently, it was shown that H-ras activation (a signaling pathway element in cells) is an early event in the ptaquiloside-induced carcinogenesis.[222,223]

Two other cyclopropanoid compounds with potential against human diseases such as cancer have been reported by Marquez et al.[5] Curacins A (**190**) and D (**191**, Scheme 68) originate from marine cyanobacteria (blue-green algae).[224,225] Curacin A was found to be an inhibitor of tubulin polymerization and binds to the colchicine drug binding site. Curacin D was isolated from *Lyngbya majuscula* and is very likely synthesized by methylene transfer from SAM.[5] The involvement of cyclopropane ring opening in any of the biological activities has not yet been described. Potentially this could be nucleophilic or radical, with the thiazoline as the acceptor or electron donor, respectively.

Scheme 68



IV. Acknowledgment

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