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Review

On the origins of triterpenoid skeletal diversity

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Dedicated to Prof. E. J. Corey on the happy occasion of his 75th birthday

Abstract

The triterpenoids are a large group of natural products derived from C_{30} precursors. Nearly 200 different triterpene skeletons are known from natural sources or enzymatic reactions that are structurally consistent with being cyclization products of squalene, oxidosqualene, or bis-oxidosqualene. This review categorizes each of these structures and provides mechanisms for their formation.

Keywords: Triterpene; Triterpenoid; Oxidosqualene cyclization; Squalene; Cyclization; Isoprene rule

Contents

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1. Introduction

The triterpenoids are a large and structurally diverse group of natural products derived from squalene (**99**) or related acyclic 30-carbon precursors (Connolly and Hill, 2002). Triterpenoids with well-characterized biological activities include sterols, steroids, and saponins. This large group of natural products displays well over 100 distinct skeletons. Most triterpenoids are 6-6-6-5 tetracycles, 6-6-6-6-5 pentacycles, or 6-6-6-6-6 pentacycles, but acyclic, monocyclic, bicyclic, tricyclic, and hexacyclic triterpenoids have also been isolated from natural sources. An unusually complex and flexible reaction mechanism generates these ring systems. In the early 1950s, Ruzicka and co-workers deduced that all $C_{30}H_{50}O$ triterpene alcohols known by that time were biosynthesized similarly, and they proposed the biogenetic isoprene rule, a set of governing principles that could explain the biosynthesis of each triterpene skeleton (Ruzicka et al., 1953; Eschenmoser et al., 1955; Arigoni, 1959; Ruzicka, 1959, 1963). As a general mechanism, all-*trans* squalene (**99**) or oxidosqualene (**1**) is activated by cationic attack. A cascade of cation–olefin cyclizations then generates a cyclic carbocation, which can rearrange and cyclize further. Antiperiplanar shifts terminated by proton loss then yield a neutral species. Several decades of research have refined the biogenetic isoprene rule. Extensive efforts in natural product isolation provided numerous additional triterpenoids, which consistently had structures that could be rationalized by the biogenetic isoprene rule, providing further support for these guidelines. Consequently, although the formation of only a minority of triterpenoid ring systems has been experimentally investigated, enzyme-mediated cyclization of squalene or oxidosqualene under the biogenetic rule is the most credible origin of these triterpenoids. The biogenetic isoprene rule predicts product structures so consistently that the plausibility of a newly assigned structure can be assessed based on whether its formation can be deduced according to the isoprene rule. This review provides a comprehensive accounting of the known natural compounds that are plausible enzymatic cyclization products of squalene (**99**), oxidosqualene (**1**), and bis-oxidosqualene (**166**).

The enzymes that catalyze these reactions are collectively known as triterpene synthases and can be

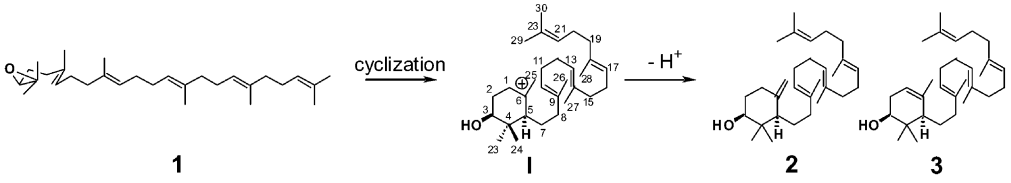

Fig. 1. Cyclization of oxidosqualene (**1**) to generate monocyclic triterpenes from cation **I**.

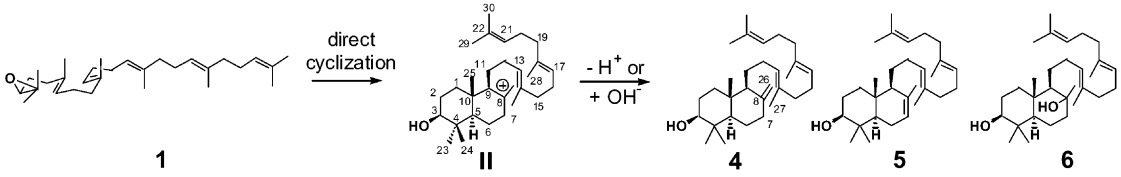

Fig. 2. Cyclization of oxidosqualene (**1**) to form bicyclic triterpene compounds from bicyclic cation **II**.

categorized as squalene cyclases (SCs; terpene synthase g, tpsg) or oxidosqualene cyclases (OSCs; terpene synthase h, tpsh), which convert squalene (**99**, Fig. 21) and oxidosqualene (**1**, Fig. 1), respectively, to cyclic triterpenes and triterpene alcohols. SCs and OSCs catalyze reactions that are mechanistically similar but phylogenetically distinct from terpene synthases a–f, which convert the acyclic allylic diphosphates geranyl pyrophosphate, farnesyl pyrophosphate, and geranylgeranyl pyrophosphate to monoterpenes, sesquiterpenes, and diterpenes, respectively (Bohlmann et al., 1998; Trapp and Croteau, 2001). Two triterpene synthases have been accessible enough to support extensive study of their mechanistic details. Lanosterol synthase is readily available from native mammalian liver or yeast, and extensive work has established that lanosterol (**12**) is biosynthesized according to the biogenetic isoprene rule (Corey et al., 1966; Abe et al., 1993; Abe et al., 1994; Wendt et al., 2000a, 2000b). Squalene-hopene cyclase (SHC) (Kannenberg and Poralla, 1999) is the most accessible SC; a recombinant expression source (Ochs et al., 1992) has provided material for structural work (Wendt et al., 1997; Wendt et al., 1999) and mechanistic study (Wendt et al., 2000a, 2000b; Hoshino and Sato, 2002).

Although the activity in crude homogenates is often low, native sources have provided enzymes that established an oxidosqualene origin for β-amyrin (**52**) (Corey and Ortiz de Montellano, 1967), cycloartenol (**16**) (Benveniste and Massy-Westropp, 1967), protosta-17(20),24-dien-3-ol (Kawaguchi et al., 1973), lupeol (**41**) (Barton et al., 1975), cucurbitadienol (Balliano et al., 1983), α-amyrin (**51**) (Abe et al., 1989), parkeol (**14**) (Makarieva et al., 1993), isomultiflorenol (**56**) (Cho et al., 1993), dammarenediol (**21**) (Kushiro et al., 1997), and friedelin (**61**) (Corsino et al., 2000). More recently, recombinant sources have confirmed that taraxasterol (**63**) (Segura et al., 2000), ψ-taraxasterol (**62**) (Segura et al., 2000), 3,20-dihydroxylupane (Segura et al., 2000), bauerenol (**69**) (Kushiro et al., 2000a), multiflorenol (**55**) (Kushiro et al., 2000), and isomultiflorenol (**56**) (Hayashi et al., 2001a) are OSC products. Novel squalene cyclization products have also been generated by mutant forms of recombinant squalene-hopene cyclase (Hoshino and Sato, 2002). To better understand the biosynthesis of triterpenoid ring systems and the cyclase enzymes, the origins of more than 100 putative squalene, oxidosqualene, and bis-oxidosqualene cyclization products are rationalized here under the biogenetic rule and categorized according to the proposed cyclization mechanisms. In the following discussion, cyclic cation intermediates that determine the ring structures of the final products are denoted with Roman numerals. The conformation of six-membered rings in the triterpenoids and the cationic intermediates are described as chair (C) or boat (B) structures.

2. Oxidosqualene cyclization

Oxidosqualene (**1**) was first shown to be the immediate precursor of lanosterol (**12**, Fig. 4) (Corey et al., 1966) and has since been enzymatically converted to a variety of triterpene alohols. Oxidosqualene (**1**) is probably the precursor of most 3β-OH-triterpenoids, although squalene cyclization followed by oxidation at C3 is also plausible. Groups that are *trans* in the acyclic olefins are typically *anti* in cyclic terpenes. Ruzicka and co-workers proposed that this stereochemical information was maintained via well-defined bridged (non-classical), short-lived, ionic intermediates (Ruzicka et al., 1953; Eschenmoser et al., 1955; Arigoni, 1959; Ruzicka, 1959, 1963). Cyclization and rearrangement reactions were postulated to be non-stop to preclude the participation of stable intermediates derived from the ionic species through deprotonation and/or hydration.

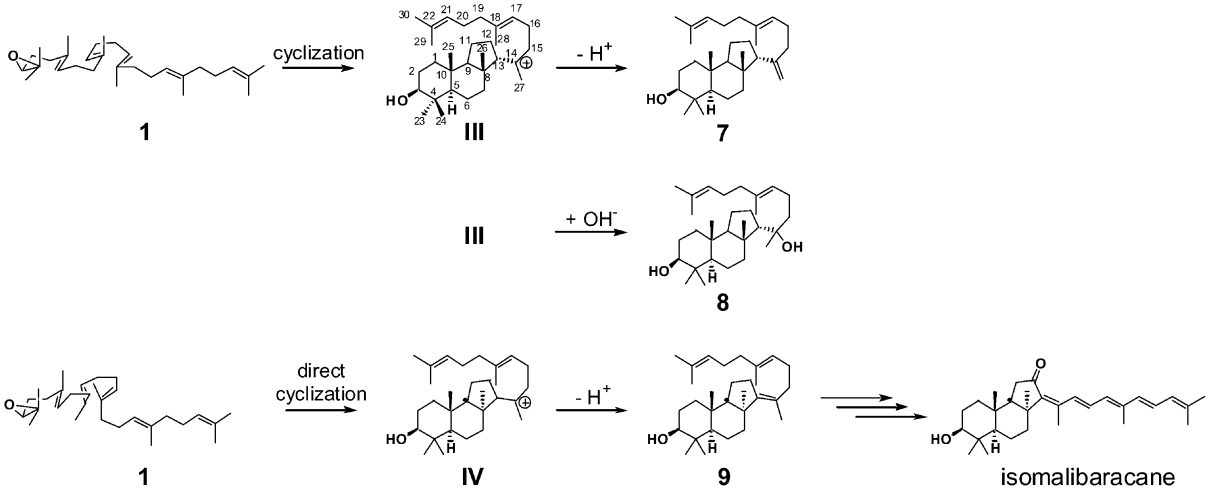

Fig. 3. Cyclization of oxidosqualene (**1**) to generate tricyclic triterpene alcohols from the tricyclic cations **III** and **IV**.

Whether the initial cyclizations are concerted (Seemann et al., 2002) or nonconcerted (Corey and Cheng, 1996; Jenson and Jorgensen, 1997; Hoshino and Sakai, 1998) remains an actively discussed topic. After initial cyclization, ring expansion and/or further annulation can generate a modified ring system. Rearrangement generally occurs through 1,2 shifts, but 1,3 shifts have been detected in sesquiterpene biosynthesis (Masciadri et al., 1985; Cane and Tandon, 1995) and are occasionally invoked for the formation of specific triterpenes (Ayoub and Babiker, 1984; Shiojima et al., 1989b; Chakravarty et al., 1991). The observed shifts are generally antiperiplanar, but a *syn* 1,2 shift has been detected in lanosterol biosynthesis (Corey and Virgil, 1991), and OSC products such as cucurbitadienol (Balliano et al., 1983) require the functional equivalent of a formal *syn* shift. Stable structures usually arise by deprotonation to $C_{30}H_{50}O$ triterpene alcohols, although alternatively, the carbocation may be quenched with water to yield $C_{30}H_{52}O_2$ diols (Kushiro et al., 1997; Meyer et al., 2000a, 2000b).

2.1. Monocyclic triterpene alcohols

The simplest OSC products are the $C_{30}H_{50}O$ monocyclic alcohols achilleol A (**2**) (Barrero et al., 1989) and camelliol C (**3**, Fig. 1) (Akihisa et al., 1999), both of which result from abstracting different protons from carbocation **I**. These monocycles are isolated from Asteridae subclass members *Achillea odorata* and *Camellia sasanqua*. Although neither achilleol synthase nor camelliol synthase has been cloned from a native source, camelliol C has been produced in the laboratory by mutants of cycloartenol synthase (Matsuda et al., 2000), and achilleol A can also be produced by mutants of either lanosterol synthase (Joubert et al., 2000) or cycloartenol synthase (Matsuda et al., 2000; Meyer et al., 2002; Segura et al., 2002).

2.2. Bicyclic triterpene alcohols

The known bicyclic $C_{30}H_{50}O$ triterpene alcohols are C–C, *trans*-decalin derivatives. These compounds have not been experimentally shown to be OSC products, but no plausible alternative origin has been proposed. In oxidosqualene (**1**) cyclization, epoxide opening is concerted with A-ring formation (Corey et al., 1997; Gao et al., 1998), and the formation of the B-ring is probably also concerted. Deprotonation of H26 or H7 from carbocation **II** (Fig. 2) generates polypoda-8(26),13,17,21-tetraen-3-ol (**4**) (Bennett et al., 1993) or polypoda-7,13,17,21-tetraen-3-ol (**5**) (Nguyen and Harrison, 1998), both of which were found in *Cratoxylum cochinchinense*. A second way to generate a neutral product is to quench the C8 carbocation with a hydroxide equivalent to generate the $C_{30}H_{52}O_2$ *Pistacia* component polypoda-13,17,21-trien-3,8-diol (**6**, Fig. 2) (Boar et al., 1984). However, the possibility that a separate enzyme hydrates **4** or **5** to yield **6** cannot be precluded. All three tricyclic OSC products are accessible by neutralizing carbocation **II** (either by deprotonation or water addition) without rearrangement. It should be emphasized that the biosyntheses of these compounds have not been experimentally verified, but the proposed origins are consistent with established precedent from the biosynthesis of other triterpene

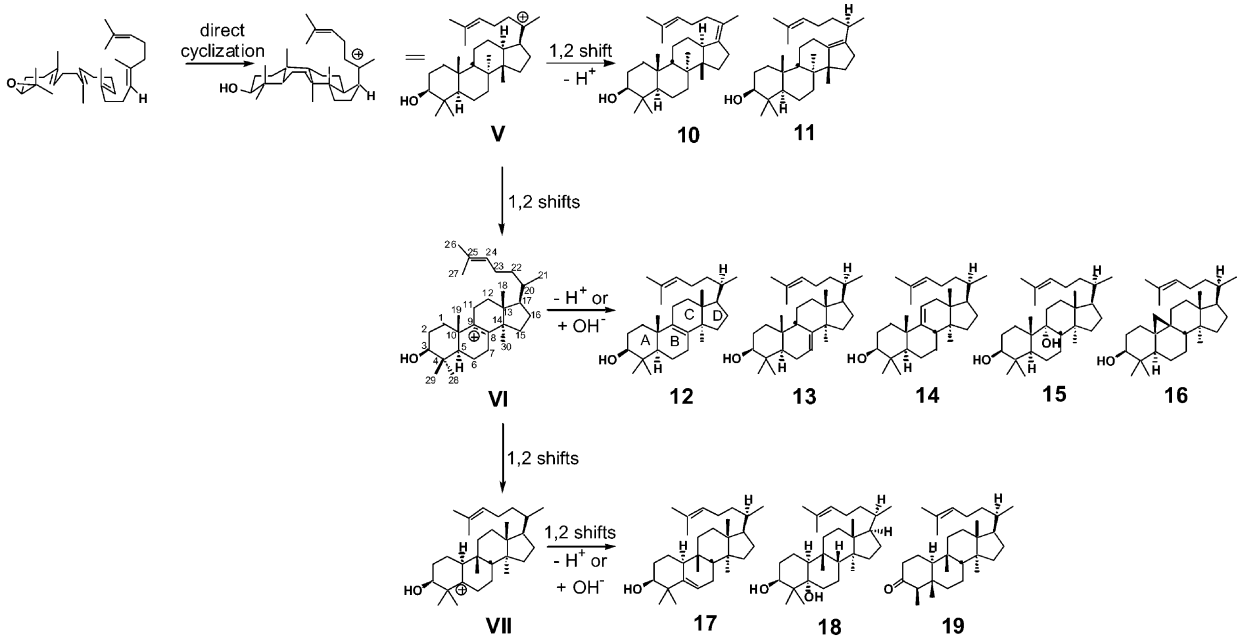

Fig. 4. Tetracyclic triterpene alcohols **10**–**19** are derived from the protosteryl cation (**V**), the lanosteryl cation (**VI**), or the cucurbitenyl cation (**VII**).

alcohols; similar caveats apply to most of the compounds in this review.

Bicyclic triterpene alcohols have limited taxonomic distribution; both *Cratoxylum* and *Pistacia* are members of subclass Rosidae. Although B-ring boats are well-represented in triterpenoids with three or more rings, enzymatically produced bicyclic triterpene alcohols derived from a C-B bicyclic cation (which would be recognizable by their 9-β-H atom) are unknown.

2.3. Tricyclic triterpene alcohols

Malabarica-14(27),17,21-trien-3-ol (Jakupovic et al., 1987) **7** is generated by cyclizing oxidosqualene to the Markovnikov-favored 6-6-5 (C–C) tricyclic tertiary carbocation **III**, followed by deprotonation from C27 (Fig. 3). Like the bicyclic structures **4–6**, tricycle **7** is consistent with concerted cyclization, followed by deprotonation without rearrangement. The tricyclic diol malabarica-17,21-dien-3,14-diol (**8**) derived from quenching cation **III** with a hydroxide equivalent has not been found in nature but has been generated in the laboratory by the *A. acidocaldarius* squalene-hopene cyclase (SHC) F601A mutant (Hoshino and Sato, 2002). Although no $C_{30}H_{50}O$ triterpene alcohol derived from the C–B 6-6-5 tricyclic cation **IV** has been isolated, sponge compounds known as isomalibaracenes are probably metabolites of the C–B tricycle **9** (Fig. 3) (McCabe et al., 1982).

2.4. Tetracyclic triterpene alcohols

OSCs generate numerous tetracyclic triterpene alcohols because the tetracyclic cations frequently undergo rearrangement prior to neutralization by deprotonation or water addition. Moreover, tetracyclization can produce two different intermediate cations that differ in B-ring configuration: the C–B–C protosteryl cation (**V**, Fig. 4) and the all-chair dammarenyl cation (**IX**/**X**, Fig. 6).

2.4.1. Tetracyclic 6-6-6-5 triterpene alcohols derived from the protosteryl cation: protostane, lanostane, cucurbitane, and related skeletal types

The biosynthesis of the tetracyclic triterpene alcohols derived from the protosteryl cation (**V**) has been studied intensively because these compounds are the initial cyclic intermediates in sterol biosynthesis. Compounds that retain the C–B–C ring system result from direct deprotonation of the protosteryl cation (**V**) either without rearrangement or with only a 17α hydride shift. The corresponding triterpene alcohols, protosta-17(20),24-dien-3-ol (**10**) (Hattori et al., 1969; Tiwari and Choudhary, 1981) and protosta-13(17),24-dien-3-ol (**11**) (Hattori et al., 1969), are known from the fungus *Cephalosporium caerulens*. Oxygenated triterpenoid derivatives of **10** or **11** were also isolated from *Fusidium coccineum* and *Aspergillus fumigatus*, suggesting that diverse members of Ascomycota can cyclize oxidosqualene to C–B–C protostane derivatives.

Cation **V** is the cyclic intermediate en route to the physiologically important steroidal triterpenes, which are precursors of sterols and steroids. A cascade of 1,2-hydride and methyl shifts generates the lanosteryl cation (**VI**), which undergoes deprotonation at C8, C11, or C19 to form the sterol precursors lanosterol (**12**) (Curtis et al., 1952; Barnes et al., 1953a,b), parkeol (**14**) (Schreiber and Osske, 1964), and cycloartenol (**16**) (Bentley et al., 1953). Although cycloartenol (**16**) has five rings, it is classified here with lanosterol (**12**) and parkeol (**14**) because these three compounds are formed by nearly identical mechanisms. Experiments with an oxa-substrate analog showed that the protosteryl cation **V** side chain is in the 17β orientation and a 60° rotation of the side chain around the C-17–C-20 bond is required prior to the antiperiplanar 1,2 hydride migration from C-17 to C-20 (Corey and Virgil, 1991; Corey et al., 1991, 1995). Lanosterol (**12**) is the initial carbocyclic intermediate in the biosynthesis of cholesterol in mammals and ergosterol in fungi. Sea cucumbers cyclize oxidosqualene (**1**) to parkeol (**14**) en route to sterols (Cordeiro et al., 1988; Cordeiro and Djerassi, 1990; Kerr and Chen, 1995), and parkeol is also a widespread plant metabolite. Cycloartenol (**16**) is the sterol precursor in plants and some protists (Benveniste et al., 1966; Rees et al., 1969; Brandt et al., 1970; Raederstorff and Rohmer, 1985, 1986, 1987; Nes et al., 1990). Further methyl and hydride shift of lanosteryl cation **VI** yields cucurbitenyl cation **VII**, the precursor of the experimentally established OSC product cucurba-5,24-dienol (**17**) (Itoh et al., 1980; Balliano et al., 1983). Litsomentol (**18**) (Govindachari et al., 1971), and nigrum-21-3-one (**19**) (Toiron et al., 1995) are angiosperm triterpenes generated by further rearrangement from cation **VII** (Fig. 4).

The cyclization of oxidosqualene (**1**) to cation **V** was originally considered to be concerted because methyl and hydrogen groups that are *trans* in squalene (**99**) remain *anti* in natural lanostane derivatives. However, a

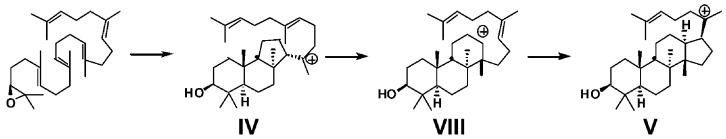

Fig. 5. Protosteryl cation **V** may arise from initial cyclization to 6-6-5 tricyclic cation **IV** and ring expansion to 6-6-6 tricyclic cation **VIII**.

growing body of experimental and theoretical work supports the position that cyclization occurs initially to 6-6-5 tricyclic cation **IV** (Fig. 5) (Corey et al., 1995; Corey and Cheng, 1996; Jenson and Jorgensen, 1997; Hess, 2002). Ring expansion would then form cation **VIII**, and **V** would result after D-ring annulation. Whether cyclization to the protosteryl cation is concerted or non-concerted remains an actively discussed topic (Seemann et al., 2002).

The OSCs that generate products from the protosteryl cation are the best studied subclass, and many genes that encode OSCs have now been cloned. Lanosterol synthases have been cloned from fungi including *Candida albicans* (Kelly et al., 1990; Roessner et al., 1993), *Saccharomyces cerevisiae* (ScERG7) (Corey et al., 1994), *Schizosaccharomyces pombe* (Corey et al., 1996; Shi et al., 1994), *Cephalosporium caerulens* (Abe et al., 2001), and *Pneumocystis carinii* (Milla et al., 2002); mammals including rat (Abe and Prestwich, 1995; Kusano et al., 1995) and *Homo sapiens* (Baker et al., 1995; Sung et al., 1995); and the protists *Trypanosoma brucei* (Buckner et al., 2000) and *Trypanosoma cruzi* (Joubert et al., 2001). Cycloartenol (**16**) is a common component in many plant oils. Cycloartenol synthases have been cloned from the angiosperm plants *Arabidopsis thaliana* (Corey et al., 1993), *Pisum sativum* (Morita et al., 1997), *Panax ginseng* (Kushiro et al., 1998a), *Luffa cylindrica* (Hayashi et al., 1999), *Glycyrrhiza glabra* (Hayashi et al., 2000), the slime mold *Dictyostelium discoideum* (Godzina et al., 2000), and the bacterium *Stigmatella aurantiaca* (Bode et al., 2003).

Parkeol synthase has not yet been cloned, but enzymes that produce parkeol (**14**) have been generated in the laboratory. Mutant cycloartenol synthases (Hart et al., 1999; Herrera et al., 2000; Matsuda et al., 2000; Meyer et al., 2002; Segura et al., 2002) and lanosterol synthases (Meyer et al., 2000a, 2000b) have been shown to convert oxidosqualene (**1**) to parkeol (**14**). Enzyme mutagenesis studies have generated two novel lanostane skeletons that have not been found in nature. 9β-Lanosta-7,24-dien-3β-ol (**13**) is produced by *A. thaliana* cycloartenol synthase (AtCAS1) mutants (Herrera et al.,

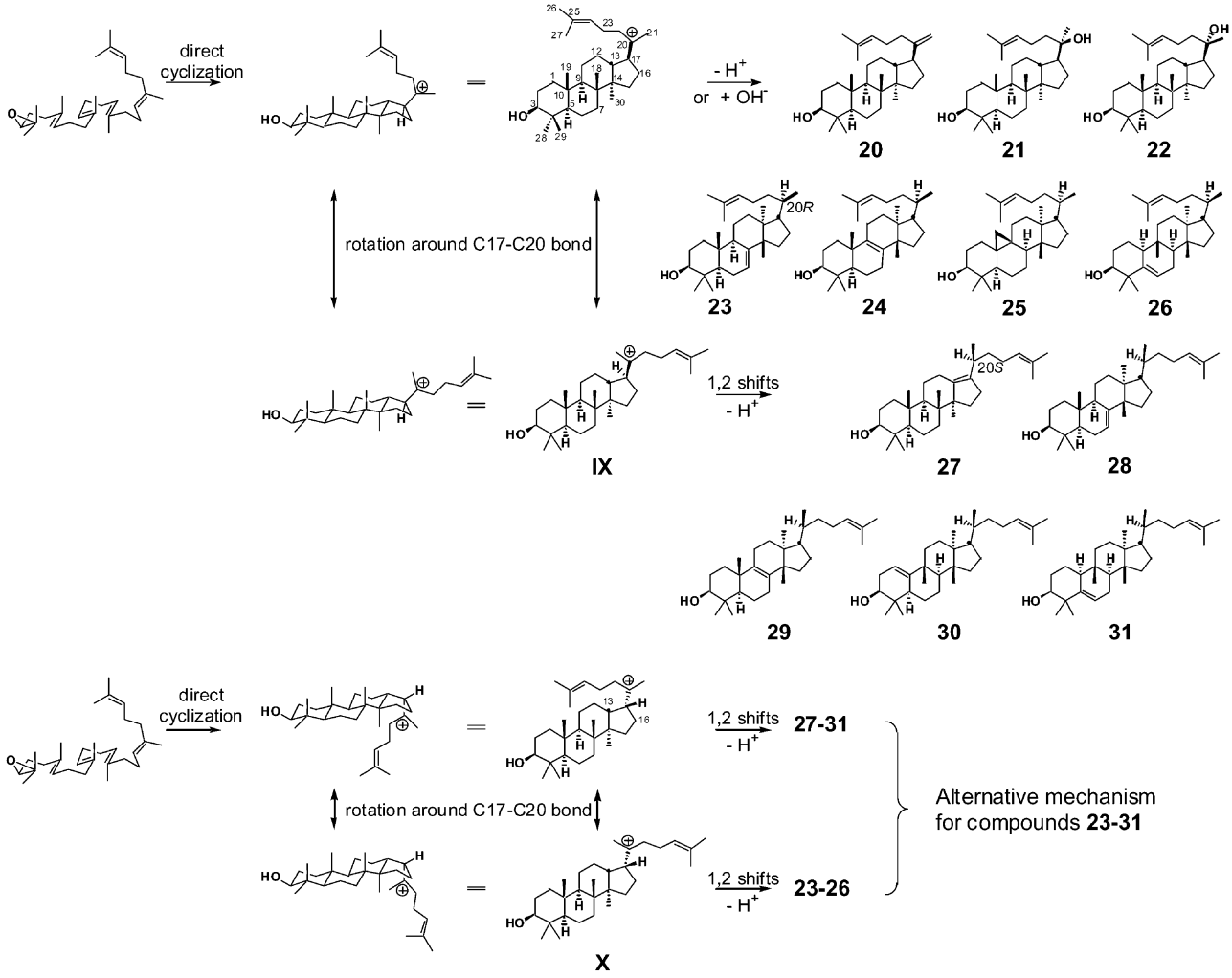

Fig. 6. Tetracyclic 6-6-6-5 triterpene alcohols derived from the dammarenyl cation.

2000; Meyer et al., 2002; Segura et al., 2002), and a modification of *S. cerevisiae* lanosterol synthase allows water to quench the C9 cation to form lanost-24-ene-3β,9β-diol (**15**) (Meyer et al., 2000a, 2000b).

2.4.2. Tetracyclic 6-6-6-5 triterpene alcohols derived from the dammarenyl cation: dammarane, euphane, tirucalane, and related skeletal types

A large variety of triterpene alcohols arise from one of two epimeric dammarenyl cations, 17β-dammarenyl cation **IX** or 17α-dammarenyl cation **X**, which are 6-6-6-5 tetracycles with all-chair configurations. Cyclization to dammarenyl cations is generally drawn as concerted, although by analogy with the protosteryl cation **V**, initial cyclization might form a 6-6-5 tricycle, followed by ring expansion and D-ring annulation. Dammara-17(20),24-dien-3-ol (**20**) (Mills, 1956), (20*R*) dammarenediol (**21**) (Mills, 1956), and (20*S*) dammarenediol I (**22**) (Mills, 1956) are generated by direct deprotonation or addition of a hydroxyl group at C20 without rearrangement. The 17β stereochemistry in compounds **20–22** is consequently derived from that of the dammarenyl cation intermediate, which consequently must have the structure of **IX**. In contrast, the $C_{30}H_{50}O$ dammarane derivatives **23–31** could arise from either C-17 epimer (**IX** or **X**) of the dammarenyl cation. These rearranged tetracyclic dammarane derivatives fall into two groups: the 20*R* compounds butyrospermol (**23**) (Irvine et al., 1956; Pradhan and Khastgir, 1969), euphol (**24**) (Arigoni et al., 1954; Barton et al., 1954; Barton et al., 1955), cycloroylenol (**25**) (Vijaya et al., 1982), and boeticol (**26**) (Ferreira et al., 1995); and the 20*S* compounds dammara-13(17),24-dien-3-ol (**27**) (Mata et al., 1991; Akihisa et al., 1997), tirucalla-7,24-dien-3-ol (**28**) (Itoh et al., 1976), tirucallol (**29**) (Arigoni et al., 1955; Menard et al., 1955), melliferol (**30**) (Ferreira et al., 1990), and euferol (**31**) (Ferreira et al., 1990). Each of these compounds can be envisioned as arising from either carbocation. Compounds **23–26** can be produced from cation **IX** if 17α-H migrates with the side chain *anti* to C16, or from cation **X** if the side chain rotates so that 17β-H can migrate with the side chain *syn* to C16 as shown in Fig. 6. Similarly, compounds **27–31** can arise from cation **IX** if 17α-H shifts with the side chain *syn* to C16 or from cation **X** if 17β-H shifts with the side chain *anti* to C16 (Fig. 6). Additional experimentation is necessary to resolve the structures of the intermediates. From cations **IX** or **X**, compounds **23–25** can be produced by a series of 1,2-shifts terminated by the elimination of H7, H9, or H19, respectively. Compound **26** results from further 1,2-shifts and H6 loss from a C5 carbocation. Compound **27** can be formed by H17 shift and H13 loss from the C17 cation. Compounds **28**, **29**, and **31** are the 20*S* epimers of the 20*R* triterpenes alcohols **23**, **24**, and **26**. As noted above, the stereochemical difference at C20 may be transferred from 17α and 17β dammarenyl carbocations **IX** and **X**. Alternatively, both C20 epimers can arise from either individual carbocation because the side chain can rotate to be *anti* or *syn* to C16 when H17 shifts to C20. Compound **30** results from a series of 1,2-shifts followed by deprotonation at C1 from the C10 carbocation. Dammarane derivatives (**23–26** and **27–30**) are unusually prevalent in the genus *Euphorbia*.

2.4.3. 6-6-6-6 Triterpene alcohols derived from the dammarenyl cation: baccharane and related skeletal types

Dammarenyl cation **IX**/**X** can undergo D-ring expansion through C16 migration to form the baccharenyl cation **XI** (Fig. 7). In principle, either C17 epimer of the dammarenyl cation could be an intermediate because the ring expansion yields a planar C18 carbocation (initially C17 in **IX**/**X**) that has lost the previous stereocenter but generates a new one at C17 (initially C20 in **IX**/**X**). Many known tetracyclic and pentacyclic triterpenoids are best rationalized as arising from the baccharenyl cation with 17*R* stereochemistry, but no known compounds are obviously derived from the 17*S* isomer. Although D-ring expansion is usually followed by E-ring cyclization to generate pentacycles (Section 2.5.2), several natural 6-6-6-6 tetracyclic triterpenes have been reported. Bacchara-12,21-dien-3-ol (**32**) (Akihisa et al., 1994), baccharis oxide (**33**) (Anthonsen et al., 1970; Mo et al., 1972), and shionone (**34**) (Takahashi et al., 1967) are generated from cation **XI** via a series of 1,2 shifts followed by different deprotonations (Fig. 7).

2.5. Pentacyclic triterpene alcohols

Pentacyclic compounds comprise the most numerous class of OSC products. Their structural variety reveals that they arise through a variety of cyclization modes. Both the protosteryl cation and the dammarenyl cation can undergo ring expansion and annulation of a fifth ring. Varying the position and facial attack of this annulation generates multiple isomeric cations that rearrange to form numerous products. Direct pentacyclization either through C–B–C–C or all-chair cations is another alternative that generates product diversity.

2.5.1. Pentacyclic C–B–C–C(–C) 6-6-6-6-5 or 6-6-6-6-6 rings: arborinane, stictane, and related skeletal types

Several pentacyclic triterpene alcohols are known that have B/C ring stereochemistries resembling those of compounds **10–19** that are derived from protosteryl cation **V**. These compounds can be rationalized as arising by D-ring expansion of **V**, followed by E-ring cyclization to form a 6-6-6-6-5 ring structure with C–B–C–C configuration. D-ring expansion via C16 migration and E-ring closure from 18α yield cation **XII**, which, after

1,2 shifts and deprotonation of H11 and H6, leads to hancolupenol (**35**) (Lou et al., 1991) and hancokinol (**36**) (Lou et al., 1991) (Fig. 8), both found in *Cynanchum hancokianum*. The stereochemistry of C18 in cation **XII** is transferred to C19 after the 18β hydride shift, resulting in 19α-isopropyl groups.

Alternatively, D-ring expansion via C13 migration and E-ring cyclization from 17β generates the arborinyl cation **XIII**, which, after 1,2 shifts, is quenched by H13 or H11 elimination to yield boehmerol (**37**) (Oyarzun et al., 1987) or isoarborinol (**38**) (Vorbrueggen et al., 1963; Kennard et al., 1967), respectively. D-ring expansion via C13 migration can also be followed by E-ring cyclization from 17α to form cation **XIV**, which undergoes 1,2 shifts and loses H11 to form sorghumol (**39**, Fig. 8). Like the rearrangement of cation **X** to **35** and **36**, the C17 stereochemical configuration in cation **XIII** and **XIV** is transferred to C21 via H17 shift, generating 21β (**37** and **38**) and 21α (**39**) isopropyl groups, respectively. Pentacyclic 6-6-6-6-5 triterpene alcohols derived from cation **V** have only been discovered in angiosperm plants to date.

Natural triterpene alcohols with C–B–C–C–C 6-6-6-6-6 rings are rare, with the sole example being stictanediol (**40**) (Chin et al., 1973) from the Ascomycota *Sticta* genus. Compound **40** is probably generated from cation **XIII** or **XIV** by the E-ring expansion (C20 migration) to yield stictyl cation **XV**, which is quenched with water to form the 20α hydroxyl group (Fig. 9).

2.5.2. Pentacyclic C–C–C–C(–C) 6-6-6-6-5 or 6-6-6-6-6 rings: lupane, germanicane, taraxastane, ursane, and other skeletal types, D-ring expansion via C16 migration followed by 18β E-ring cyclization and possible E-ring expansion

Most of the widespread triterpene alcohols in plants, such as lupeol (**41**) and β-amyrin, originate from the dammarenyl cation after D-ring expansion via C16 migration followed by 18β E-ring cyclization and, sometimes, further E-ring expansion. The lupyl cation **XVI** is generated from the dammarenyl cation (**IX** or **X**) by D-ring expansion (C16 migration) to form baccharenyl cation **XI** followed by E-ring closure to the β-face of C18. Cationic intermediate **XVI** has a *trans*-D,E ring junction. Direct deprotonation without rearrangement provides lupeol (**41**) (Ames et al., 1951). After a series of 1,2 shifts, deprotonation at different positions yields a variety of lupeol derivatives, including 18-lupen-3-ol (**42**) (Gonzalez et al., 1973), 13(18)-lupen-3-ol (**43**) (Bhan et al., 1988), neolupenol (**44**) (Ageta et al., 1981), tarolupeol (**45**) (Ageta et al., 1981), tylolupenol A (**46**) (Xu et al., 1983; Kawanishi et al., 1985), tylolupenol B (**47**) (Xu et al., 1983; Kawanishi et al., 1985), cymbopogonol (**48**) (Yokoyama et al., 1980), and cymbopogonone (**49**) (Yokoyama et al., 1980) (Fig. 10).

The five-membered E-ring in lupyl cation **XVI** can undergo expansion, either by C21 migration to form germanicyl cation **XVII** (Fig. 11) or by C18 migration to form secondary cation **XX** en route to isoursyl cation

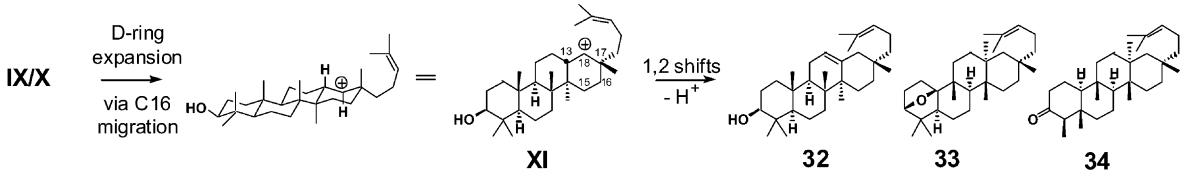

Fig. 7. Dammarenyl cation **IX** or **X** undergoes D-ring expansion via C16 migration to yield baccharenyl cation **XI**, leading to tetracyclic 6-6-6-6 triterpene compounds **32–34**.

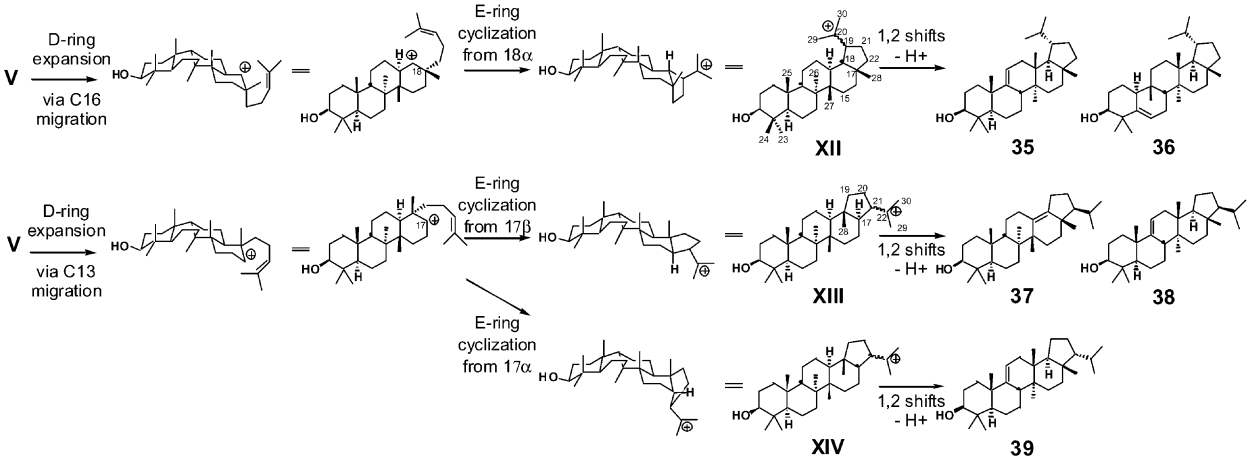

Fig. 8. The protosteryl cation **V** undergoes D-ring expansion followed by E-ring cyclization to cations **XII**, **XIII**, and **XIV** en route to pentacyclic 6-6-6-6-5 triterpene alcohols with C–B–C–C configuration.

XXI (Fig. 12). Cation **XVII** can lose a proton from C18 without rearrangement to form germanicol (**50**, Fig. 11) (David, 1949, 1950), a component of many plants. The 1,2-shifts in cation **XVII** can flow towards two directions. One direction of shifts starts with 18α-H migration, and carbocations can be generated subsequently at C18, C13, C14, C8 (taraxeryl cation), C9, C10, C5, and C3 (friedelyl cation). These cations are quenched by deprotonation or by the addition of a hydroxyl group, resulting in a variety of natural compounds including δ-amyrin (**51**) (Musgrave et al., 1952), β-amyrin (**52**) (Bischof et al., 1949), taraxerol (**53**) (Brooks, 1953), 3,14-taraxeranediol (**54**) (Hui and Li, 1976), multiflorenol (**55**) (Khastgir and Sengupta, 1961; Sengupta and Khastgir, 1963), isomultiflorenol (**56**) (Aexel et al., 1972; Hayashi et al., 2001a), walsurenol (**57**) (Chatterjee et al., 1968), dendropanoxide (**58**) (Arthur and Hui, 1961; White et al., 1973), 5-gluten-3-ol (**60**) (Fischer and Seiler, 1961), and friedelin (**61**) (Corey and Ursprung, 1956) (Fig. 11). Electron flow in the opposite direction shifts the 29-methyl group of cation **XVII** to C19 to form taraxastyl cation **XVIII**, which leads to 3,20-taraxastanediol (**61**) (Anjaneyulu et al., 1985), ψ-taraxasterol (**62**) (Ames et al., 1954), and taraxasterol (**63**) (Ames et al., 1954) by adding hydroxyl at C20 or deprotonation from C21 or C30. In the rare case of 20β-methyl group migration in cation **XVII** to cation **XIX**, 18α-20-ursen-3-ol (**64**) (Bermejo Barrera et al., 1966) is formed by deprotonation from C21 (Fig. 11).

Another group of natural products, including 18α-19(29)-ursen-3-ol (**65**) (Bhutani et al., 1992), α-amyrin (**66**) (Meisels et al., 1949, 1950), isoursenol (**67**) (Chivers et al., 1966), isobauerenol (**68**) (Talapatra et al., 1968), bauerenol (**69**) (Lahey and Leeding, 1958; Fukuoka and Natori, 1972), and rhoiptelnol (**70**) (Kitajima et al., 1994; Kiyotani et al., 1996), probably comes from isoursyl cation **XXI**. Lupyl cation **XVI** undergoes C18 migration to form **XX**, followed by C29 methyl shift to **XXI**, which either undergoes H29 elimination to form **65** or a series of 1,2 shifts and deprotonation to generate compounds **66–70** (Fig. 12).

A major obstacle to investigating triterpene cyclization is that most of these compounds are found in plants that contain multiple cyclases. Crude homogenates are consequently difficult to work with. Recent progress in cloning cyclases has provided unique access to single OSC activities, which greatly facilitates deducing the range of catalytic outcomes. Heterologously expressed lanosterol synthase and cycloartenol synthase catalyze their reactions accurately; it is apparently advantageous to biosynthesize the sterol precursors without structural diversity. Some β-amyrin synthases (Kushiro et al., 1998a, 1998b; Morita et al., 2000; Haralampidis et al., 2001; Hayashi et al., 2001b; Suzuki et

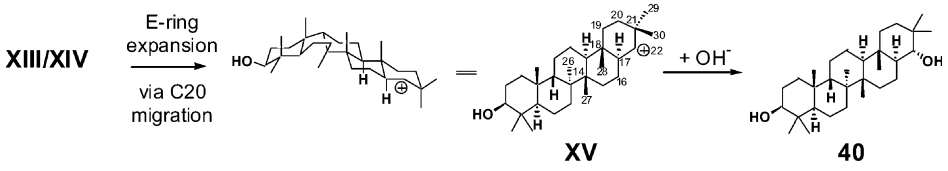

Fig. 9. Stictanediol **40** is the only known pentacyclic 6-6-6-6-6 triterpene derived from the protosteryl cation via **XIII** or **XIV**.

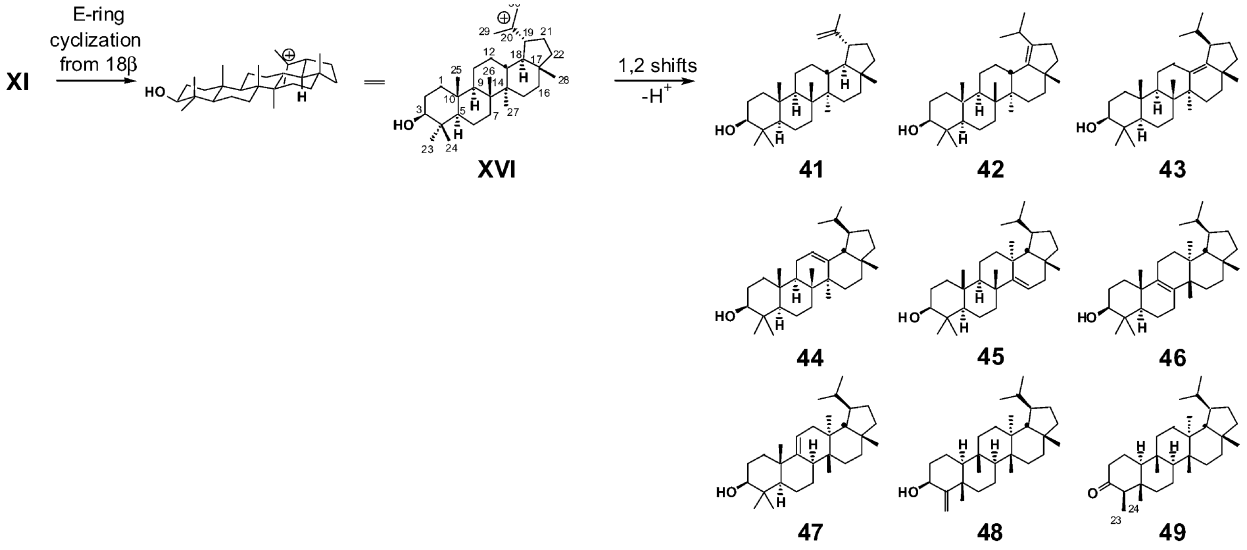

Fig. 10. Baccharenyl cation **XI** undergoes 18β E-ring cyclization to yield lupyl cation **XVI**, the precursor of lupeol (**41**) and rearranged lupenes **42–49**.

al., 2002) and some lupeol synthases (Kushiro et al., 1999; Shibuya et al., 1999) are similarly accurate and cleanly biosynthesize one product. Phylogenetic reconstructions suggest that lupeol and β-amyrin synthases, along with other known enzymes that access the dammarenyl cation, diverged from cycloartenol synthases within the plant kingdom (Herrera et al., 1998; Shibuya et al., 1999). Consequently, pentacyclic triterpenoids are ubiquitous in higher plants but nearly absent in other organisms. Mutagenesis experiments have established that single mutations can dramatically alter product profile (Segura et al., 2003). This catalytic plasticity undoubtedly contributes to the diversity of triterpenoid structures and establishes that phylogenetic similarity alone has limited utility in predicting product profile.

A further source of terpene skeletal diversity is that some enzymes are multifunctional and generate a variety of structures. Lupeol synthase from *Arabidopsis thaliana* is a multifunctional enzyme that converts oxidosqualene (**1**) to lupeol (**41**), β-amyrin (**52**), germanicol (**50**), taraxasterol (**63**), ψ-taraxasterol (**62**), and 3,20-dihydroxylupane (Herrera et al., 1998; Segura et al., 2000). A related *A. thaliana* multifunctional triterpene synthase produces lupeol (**41**), taraxasterol (**63**), β-amyrin (**52**), ψ-taraxasterol (**62**), bauerenol (**69**), α-amyrin (**57**), and multiflorenol (**55**) (Kushiro et al.,

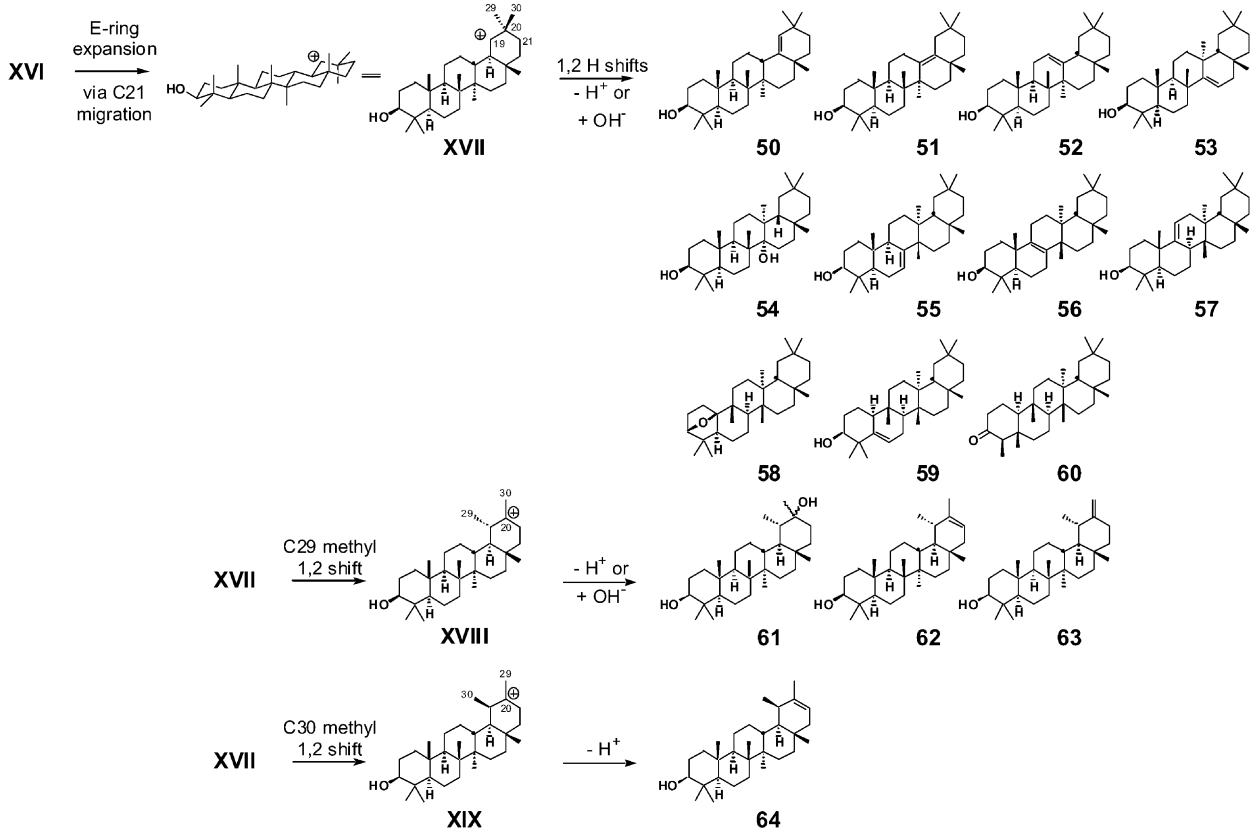

Fig. 11. E-ring expansion of lupyl cation **XVI** via C21 migration yields germanicyl cation **XVII**, leading to compounds **50–60** as well as taraxastenes **61–64**.

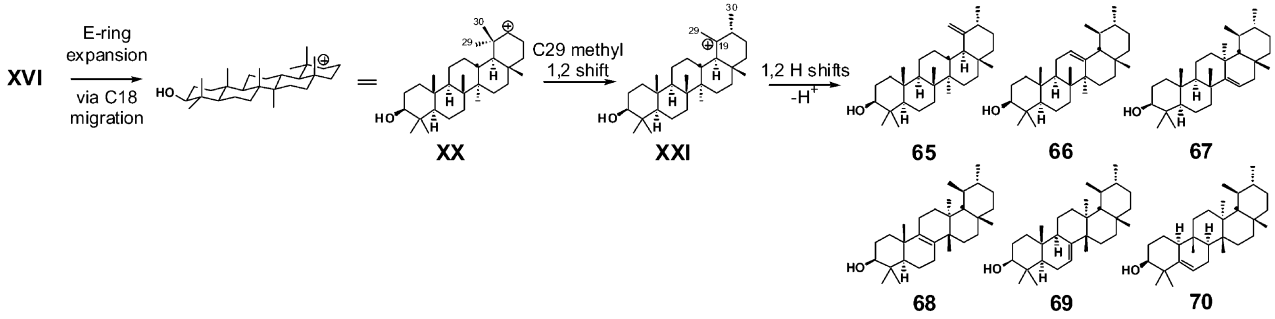

Fig. 12. E-ring expansion of lupyl cation **XVI** via C18 migration followed by C29 methyl shift yields isoursyl cation **XXI**, the probable precursor of triterpene products **65–70**.

2000a; Husselstein-Muller et al., 2001). These two enzymes apparently cyclize efficiently to the lupyl cation and allow rearrangements to both the germanicyl and taraxastyl cations, which are quenched by multiple deprotonation options or addition of a hydroxide equivalent. A *Pisum sativum* cyclase produces both α-amyrin (**66**) and β- amyrin (**52**) in approximately 3: 2 ratio, accompanied by several other minor byproducts (Morita et al., 2000). This enzyme apparently allows two different modes of E ring expansion to the germanicyl and isoursyl cations and promotes similar 1,2 shifts and proton abstraction from each cation to generate two distinct Δ12 compounds.

2.5.3. Pentacyclic C–C–C–B(–C) 6-6-6-6-5 or 6-6-6-6-6 rings, D-ring expansion via C16 migration followed by 18α E-ring cyclization and possible E-ring expansion

The catmint metabolite nepehinol (**71**, Fig. 13) (Ahmad et al., 1985) is identical to lupeol (**41**) except that the C18 position is epimerized. This stereocenter in lupeol (**41**) is established upon annulation of the E-ring from the β-face, and nepehinol (**71**) could be generated by a manner similar to lupeol (**41**) (Fig. 10) if cyclization occurred from the α-face. E-ring cyclization to 18α in cation **XI** generates the isolupyl cation **XXII** with the *cis* D–E ring junction. Direct deprotonation of H29 in cation **XXII** yields **71**.

E-ring expansion of cation **XXII** through C21 migration leads to the isooleanyl cation **XXIII** with 6-6-6-6-6 ring and C–C–C–B–C configuration. Then the migration of 30-methyl to C19 carbocation yields the C20 ursyl cation (**XXIV**), which loses H21 or H30 to form 20-ursen-3-ol (**72**) (Dominguez et al., 1974) or 20(30)-ursen-3-ol (**73**, Fig. 14) (Panosyan and Mnatsakanyan, 1977). The alternative E-ring expansion of cation **XXII** via C18 migration to the C20 cation **XXV** and subsequent C29-methyl shift yields the C19 ursyl cation (**XXVI**), the precursor of calotropenol (**74**, Fig. 14) (Khan et al., 1988). Cation **XXV** can undergo a C30 methyl shift and further 1,2-shifts followed by deprotonation to form 13(18)-ursen-3-ol (**75**) (Misra et al., 1984), phyllanthol (**76**) (Cole, 1954), and carissol (**77**) (Naim et al., 1985). Phyllanthol (**76**) is noteworthy as the sole known hexacarbocyclic oxidosqualene cyclization product. 19-Ursen-3-ol (**78**) (Misra et al., 1993) is accessible from either **XXIV**, **XXVI**, or **XXVII**. Cations

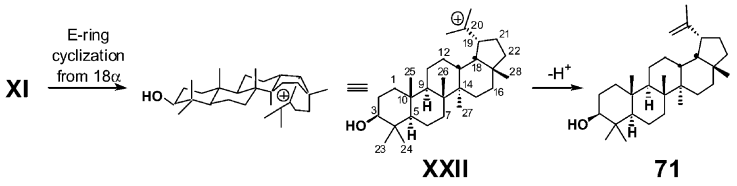

Fig. 13. The baccharenyl cation **XI** undergoes 18α E-ring cyclization to yield cation **XXII** en route to nepehinol **71**.

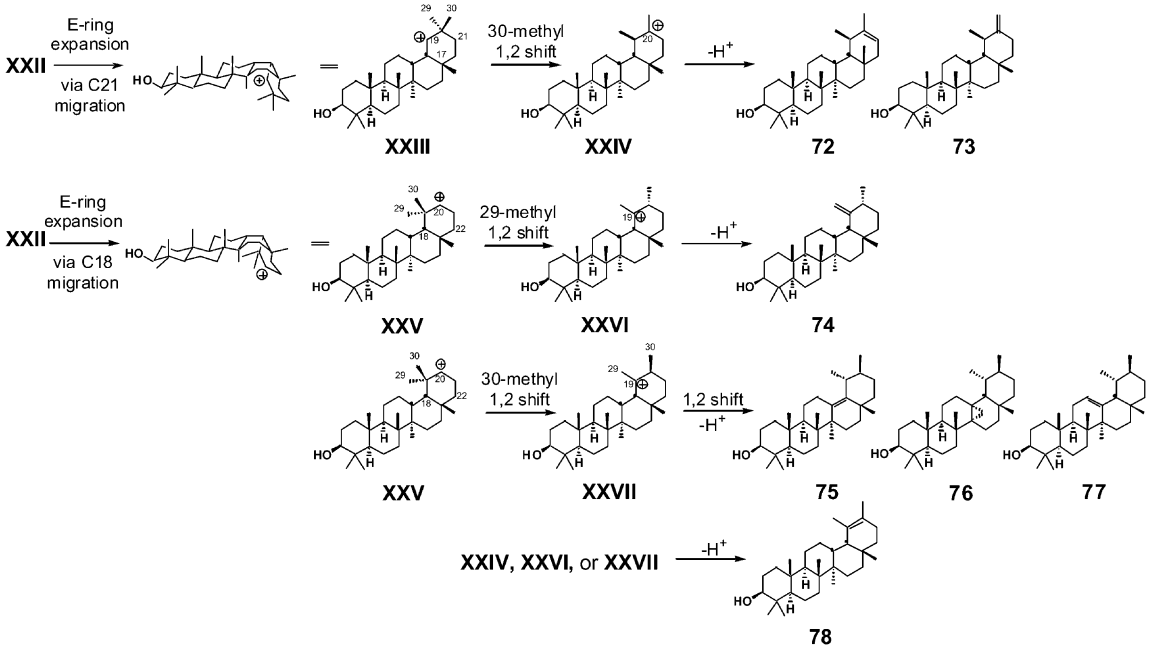

Fig. 14. E-ring expansion in cation **XXII** followed by methyl shifts yield **XXIV**, **XXVI**, or **XXVII** which are similar except that the positions of the carbocation and H-atom at C19 and C-20 are interchanged. These cations lead to ursenes **72**–**78**.

XXIV, XXVI, and XXVII are similar except that the carbocation and H-atom are positioned differently at C19 and C20.

An alternative route to ursanes 75–77 from the C19 cation XXIII is shown in Fig. 15. A β-methyl shift from C20 to C19 would yield the C20 cation XXIV, and α-hydride shift from C19 to C20 would establish the β-methyl stereochemistry at C20 and restore the cation to C19 cation XXVII, which would rearrange and undergo deprotonation as in Fig. 14. However, several difficulties in this route argue in favor of that from XXV shown in Fig. 14. It is not obvious how electronic features could promote the methyl shift/hydride shift sequence that converts XXIII to XXVII (Fig. 15). This route requires stabilizing carbocationic charge initially at C19 (XXIII) over C20 (XXV). The methyl shift to form XXIV would mandate a substrate-enzyme interaction change that transiently favors the charge at C20, and a second repositioning that again stabilizes the C19 carbocation XXVII.

Similar problems would arise if the enzyme positioned steric bulk near C19 to discourage cation XXV (which has gem-dimethyl groups on C19) formation in favor of cation XXIII formation (with the gem-dimethyl at C20). However, the active site would need space above C19 to accommodate the β-methyl generated in the conversion of XXIII to XXVII, and space below the ring is necessary to allow the inversion of that center to an α-methyl in the subsequent rearrangement en route to 75. In contrast, the route through XXV (Fig. 14) could be guided by steric bulk below C20 that would block positioning an α-methyl group there, thereby precluding the formation of cation XXIII. This motif could similarly prevent C29 from shifting to the α-position of C20. A shift of C30 to the β-position of C20 would be the sole mode to generate a more stable tertiary carbocation.

2.5.4. Pentacyclic C–C–C–C 6-6-6-6-5 rings via a spiro-fused 5,5 D–E ring intermediate

Pentacyclic triterpene alcohols are usually derived from the dammarenyl cation by D-ring expansion and E-ring cyclization to form the 6-6-6-6-5 ring structure. The friedomadeirenes are an interesting group of triterpene alcohols in which E-ring cyclization apparently precedes D-ring expansion. Triterpene alcohols D-friedomadeir-14-en-3-ol (79) (Ferreira et al., 1991) and D:C-friedomadeir-7-en-3-ol (80) (Ferreira et al., 1991) have unique structures that can be deduced from the dammarenyl cation through the following mechanism. First, H17 hydride shift from the dammarenyl cation generates the C17 cation and 20*S* stereocenter. E-ring closure between the C17 cation and the Δ24 olefin forms spiro-fused D,E five-membered rings. After another hydride shift from C21 to C22, the D ring undergoes expansion by C16 migration to form cation XXVIII which has 6-6-6-6-5 rings with C–C–C–C configuration and the unique 17β-isopropyl group. After several 1,2 shifts, the carbocationic center in cation XXVIII relocates to C14 and then to C8. Deprotonation at C15 and C7 generates final products 79 and 80 (Fig. 16), both found in *Euphorbia mellifera*.

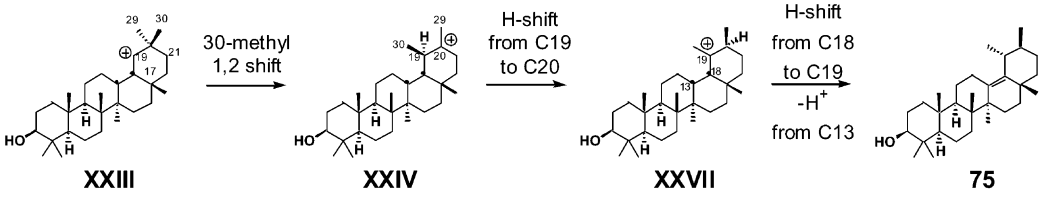

Fig. 15. A route to ursanes such as 75 from cation XXIII entails more bond changes than that from cation XXV (Fig. 14). This sequence mandates enzymatic motifs that stabilize two structurally distinct C19 carbocations at different times in the rearrangement sequence.

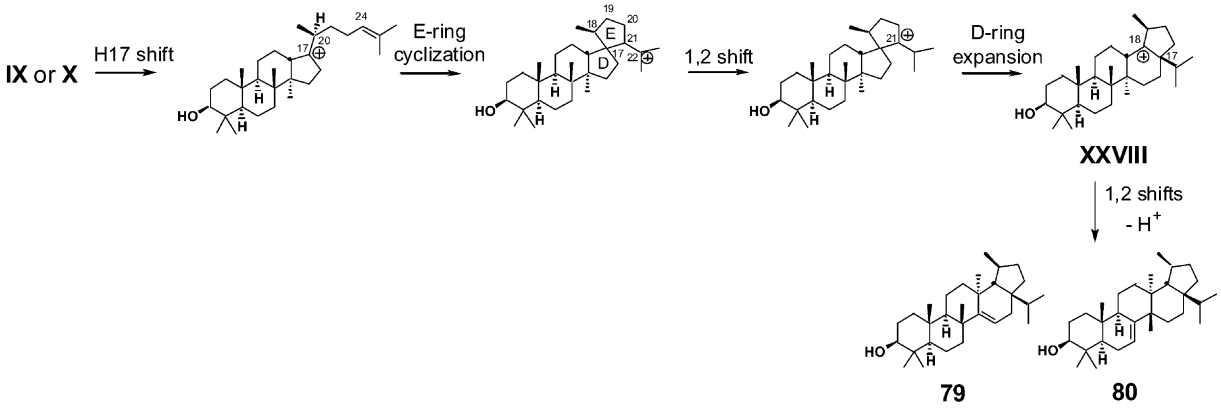

Fig. 16. In rare cases, pentacyclic triterpene alcohols are derived from dammarenyl cation via a spiro D–E ring intermediate cation XXVIII, as in the formation of triterpenes alcohols 79 and 80.

2.6. Pentacyclic C–C–C–C(–C) 6-6-6-6-5 or 6-6-6-6-6 rings: 3-hydroxyhopane, moretane, tetrahymane, and other skeletal types that are structurally consistent with direct cyclization

To correctly position methyl groups, routes to the pentacyclic compounds in **5b** and **5c** require rearrangement steps during the cyclization process. However, several natural triterpene alcohols have substitution patterns consistent with arising from concerted pentacyclization of oxidosqualene (**1**) (Fig. 17). For example, direct cyclization of oxidosqualene (**1**) to a 6-6-6-6-5 pentacyclic carbocation would position all methyl groups and hydrogen atoms at the correct position and in the appropriate *trans* orientation for deprotonation to hopenol B (**81**).

An alternative to the concerted cyclizations to cations **XXIX** and **XXXI** is cyclization to the dammanrenyl cation followed by D-ring expansion (by C13 migration rather than the C16 migration in Sections 2.4.3, 2.5.1, and 2.5.2) to the 18*S* 6-6-6-6 tetracycle **XXX** (18β side chain), in which the C19 carbocation attacks Δ24 to form the five-membered E-ring. The 21-isopropyl group on the E-ring can either adopt the β-configuration (e.g., hopyl cation **XXIX**) or the α-configuration (e.g., moretyl cation **XXXI**). Whether cyclization to these compounds is concerted or nonconcerted has not been experimentally investigated; cyclizations are shown stepwise because this mode is more readily depicted. Hopenol B (**81**) (Matsunaga and Morita, 1983) is formed from the hopyl cation **XXIX** by direct deprotonation at C30, and thus preserves the 21*R* stereochemical configuration. Moretenol (**82**) (Galbraith et al., 1965) similarly arises from the moretyl cation **XXXI** and retains the 21*S* stereocenter. Leptadenol (**83**) (Noor et al., 1993) is probably derived from cation **XXXI** through a C22–29–30 cyclopropyl cation and also retains the 21*S* stereocenter (Fig. 18). However, the H21 hydride shift in cation **XXIX** or **XXXI** usually destroys the original stereochemical configuration at C21. 17β-H elimination after an H21 shift generates 17(21)-hopenol (**84**) (Arthur et al., 1964). Alternatively, the 17β-H can undergo hydride shift to create a new stereocenter at C21 and a carbocation at C17. Further 1,2 shifts relocate the carbocation to C18, C8, C9, C5, or C3, and the respective intermediates are the precursors of 3,18-neohopanediol (**85**) (Achari et al., 1975), neomotiol (**86**) (Nakamura et al., 1965), fernenol (**87**) (Nishimoto et al., 1968), motiol (**88**) (Kariyone et al., 1957; Nakamura et al., 1965), isomotiol (**89**) (Singh et al., 1978), simiarenol (**90**) (Arthur et al., 1965; Aplin et al., 1966), and filican-3-one (**91**) (Verpoorte, 1978) (Fig. 18). The 21*R* configuration in compounds **85–91** is transferred from C17 and should be distinguished from the original 21*R* stereocenter as in hopenol B (**81**).

Similar to 6-6-6-6-5 ring cations **XVI** and **XXII**, cation **XXIX** or **XXXI** can undergo E-ring expansion via either C17 migration or C20 migration, resulting in tetrahymyl cation **XXXII** or gammaceryl cation **XXXIV**. Cation **XXXII** can be quenched directly by the addition of a hydroxide equivalent from the α-face of C21 to form 3,21-gammaceranediol (**92**, Fig. 19)

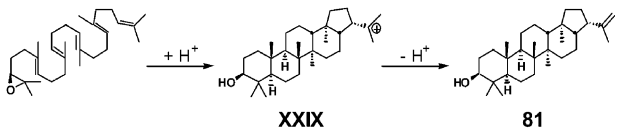

Fig. 17. The hopyl cation **XXIX** could form by direct cyclization of oxidosqualene.

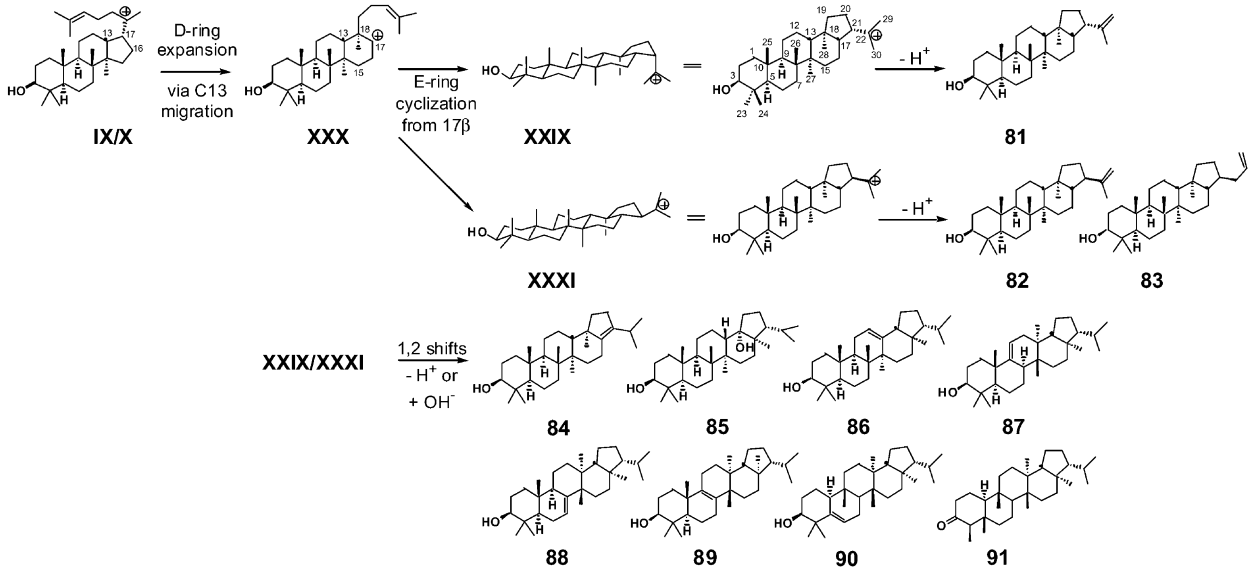

Fig. 18. Dammarenyl cation **IX**/**X** undergoes D-ring expansion via C13 migration followed by 18β E-ring cyclization to yield the hopyl cation **XXIX** or moretyl cation **XXXI**, which can rearrange to diverse products with 3-hydroxyhopane skeletons.

(Tanaka and Matsunaga, 1992). Although most natural triterpene alcohols derived from the dammarenyl cation are found in angiosperms, compound **92** has only been found in the gymnosperm *Abies veitchii*. The cyclization of oxidosqualene to 16-gammaceren-3-ol (**93**) (Shiojima et al., 1989b) would require cation **XXXII** to undergo a unique 1,3 shift to yield C17 carbocation **XXXIII** followed by H16 elimination. Cation **XXXIII** can undergo another 1,3 13β-hydride shift to C17 followed by proton loss at C12 to form monechmol (**94**) (Ayoub and Babiker, 1984). Cation **XXXIII** can also rearrange by further 1,2 shifts to yield a carbocation at C8 or C9 to form the precursors of isopichierenol (**95**) (Shiojima et al., 1989b), pichierenol (**96**) (Shiojima et al., 1989b), and swertenol (**97**, Fig. 19) (Chakravarty et al., 1991). Although these mechanisms are somewhat unorthodox because all other known triterpene cation intermediates arrange by 1,2 shifts, other alternatives to rationalize structures **93–97** are less credible. E-ring expansion via C20 migration in cation **XXIX** or **XXXI** to yield cation **XXXIV** is relatively rare. In the sole known example, chiratenol (**98**) (Chakravarty et al., 1990) apparently derives from **XXXIV** by 17β-hydride shift followed by loss of H16 (Fig. 19).

3. Squalene cyclization

A closely related series of 3-deoxytriterpenes is generated by cyclization of squalene (**99**). *The Dictionary of Natural Products* (Buckingham, 2002) contains 57 $C_{30}H_{50}$ or $C_{30}H_{52}O$ compounds that can be rationalized from protonation and cyclization of squalene (**99**) based on the biogenetic isoprene rule. The $C_{30}H_{50}$ structures result from deprotonation of a carbocation derived from squalene (**99**) cyclization; $C_{30}H_{52}O$ compounds arise by addition of a hydroxide equivalent to an intermediate cation.

Many of these have been experimentally established as SC products. In addition, extensive study into the catalytic abilities of native and mutant derivatives of the squalene-hopene cyclase (SHC) from the thermoacidophilic bacterium *Alicyclobacillus acidocaldarius* has uncovered 10 novel squalene (99) cyclization products (Hoshino and Sato, 2002). Natural sources and in vitro incubations provide a total of 66 triterpenes apparently derived from squalene (**99**) cyclization. These are categorized here in the same manner as were the putative OSC products in Part I.

It is essential to note that the structures assigned as oxidosqualene (**1**) cyclization products could alternatively derive from squalene (**99**) followed by oxidation at C3; conversely, those assigned as squalene (**99**) cyclization products could come from oxidosqualene (**1**) with the 3-hydroxyl group being reduced after cyclization. Furthermore, it is possible for one enzyme to accept multiple substrates. Oxidosqualene (**1**) is more readily protonated than is squalene (**99**), and SCs have evolved to protonate the olefinic functional group, which is less basic than the epoxy group. Therefore, SCs can often protonate and cyclize oxidosqualene (**1**) as well. For example, in addition to cyclizing its normal substrate squalene (**99**) to tetrahymanol (**165**), tetrahymanol synthase can convert 3*S* oxidosqualene (**1**) to 3,21-gammaceranediol (**92**) (Bouvier et al., 1980) (Fig. 20).

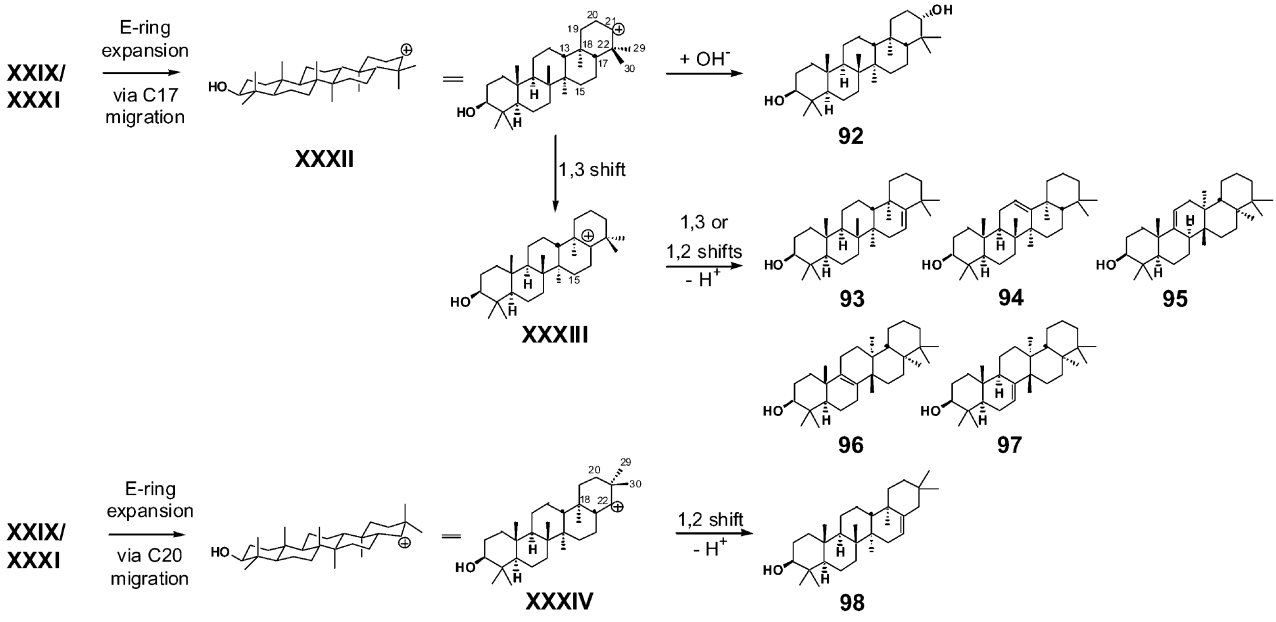

Fig. 19. The hopyl cation **XXIX** or moretyl cation **XXXI** can undergo E-ring expansion via C17 migration to generate tetrahymyl cation **XXXII**, the precursor of triterpene products **92–97**. Chiratenol (**98**) is the sole known product of a C20 migration from **XXIX** or **XXXI**.

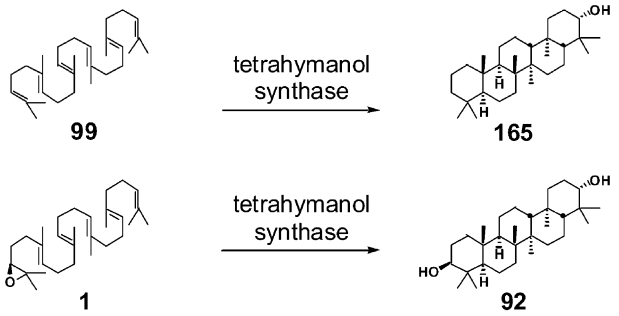

Fig. 20. Tetrahymanol synthase can cyclize either its native substrate squalene (**99**) or oxidosqualene (**1**).

However, the enzymatic cyclization of squalene (**99**) differs from that of oxidosqualene (**1**) in several respects. First, oxidosqualene is enzymatically cyclized to two distinct tetracyclic 6-6-6-5 cation intermediates with either C–C–C (dammarenyl cation) or C–B–C (protosteryl cation) configuration, and this dichotomy introduces significant structural diversity in oxidosqualene (**1**) cyclization. In contrast, known modes of squalene (**99**) cyclization only generate all-chair cations. Secondly, oxidosqualene (**1**) cyclization is always initiated from the sole terminal 2,3-epoxy group. However, squalene (**99**) is a symmetrical molecule, and some squalene (**99**) cyclization products apparently arise from two distinct cyclization events initiated from both termini (see Sections 3.1 and 3.2 below). Finally, oxidosqualene (**1**) and squalene (**99**) cyclization products have different phylogenetic distributions. Both oxidosqualene (**1**) and squalene (**99**) cyclize to key intermediates in the biosynthesis of cell membrane components. Whereas the oxidosqualene (**1**) cyclization products lanosterol (**12**), cycloartenol (**16**), and parkeol (**14**) are vital for crown eukaryotes and kinetoplastids, squalene-derived triterpenes such as hopanol and tetrahymanol (**165**) are seen in prokaryotes and ciliates. Diverse triterpenoid secondary metabolites formed by oxidosqualene (**1**) cyclization, are found primarily in angiosperm plants. In contrast, secondary metabolites derived from squalene (**99**) cyclization are mostly isolated from lower plants, including ferns and mosses.

3.1. Monocyclic triterpenes and their derivatives that originate from squalene (Fig. 21)

The monocyclic triterpene 9,13,17,21-achillatetraen-6-ol (**100**) (Arai et al., 1992) results from the proton-induced A-ring cyclization of squalene (**99**) and the subsequent aqueous quenching of the C6 carbocation **XXXV**. *A. acidocaldarius* SHC mutants have been shown to generate **100** and the related monocyclic compounds **101** and **102** (Hoshino and Sato, 2002). Monocycle **101** results from direct deprotonation, whereas **102** formation requires hydride and methyl shifts. Neither **101** nor **102** has been found in nature to date. Squalene (**99**) cyclization differs from oxidosqualene (**1**) cyclization in that the symmetric substrate can cyclize from both ends. In this case, another proton can initiate a cyclization cascade by attacking the Δ21 double bond on the other terminus of **100** to form a 6-6-5 tricycle, and the resulting carbocation is quenched by the 6α-hydroxyl group, yielding colysanoxide (**103**) (Arai et al., 1982). Like **100**, compound **101** can also be cyclized from the other terminus to generate ambrein (**104**) (Jeger et al., 1947; Oritani et al., 1970), a constituent of ambergris.

3.2. Bicyclic triterpenes and their derivatives that originate from squalene (Fig. 22)

Bicyclic natural products 7,13,17,21-polypodatetraene (**105**) (Shiojima et al., 1983), 8(26),13,17,21-polypodatetraene (**106**) (Shiojima et al., 1983), and 13,17,21-polypodatrien-8-ol (**107**) (Arai et al., 1992) can be rationalized as squalene (**99**) cyclization products derived from the C-C intermediate cation **XXXVII**

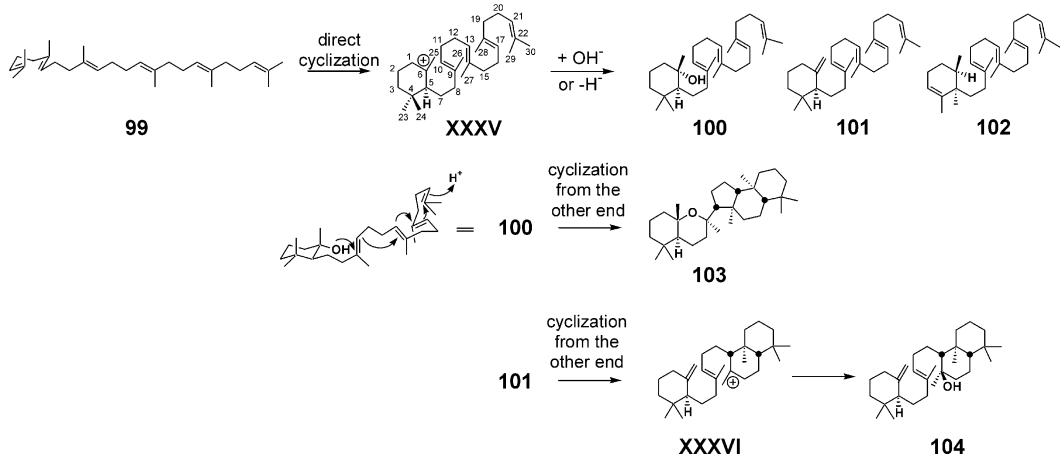

Fig. 21. Monocyclic triterpenes and derivatives that originate from squalene.

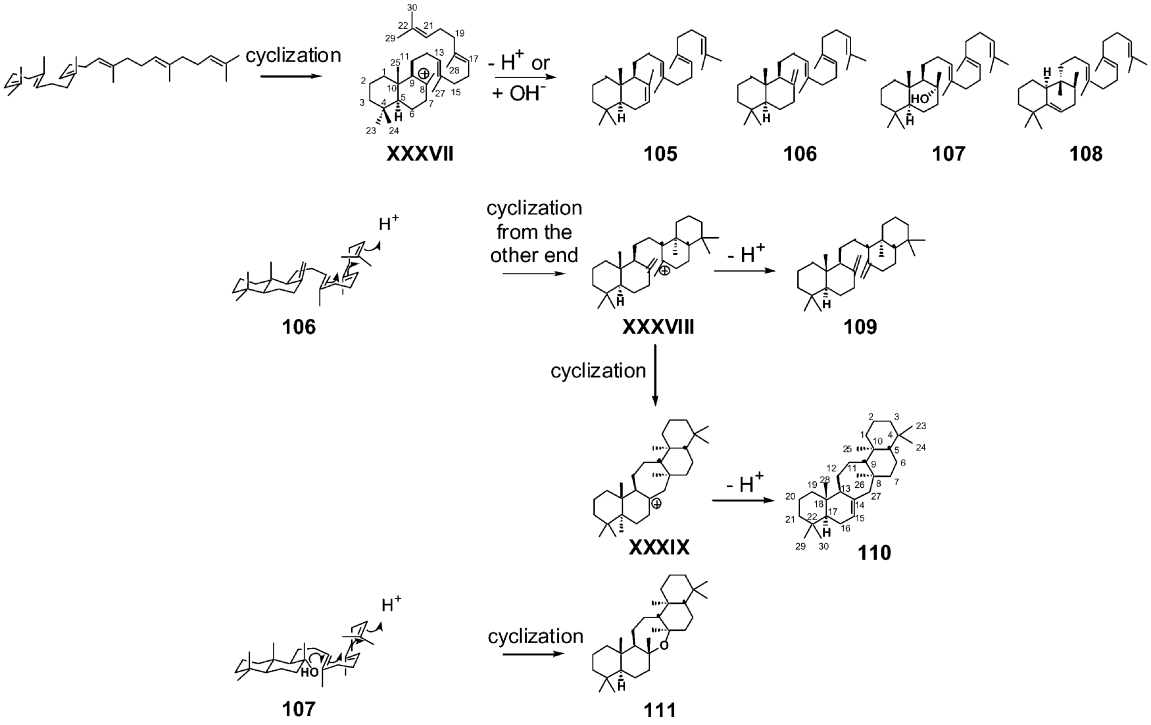

Fig. 22. Bicyclic triterpenes and derivatives that originate from squalene.

followed by immediate deprotonation or water addition. The formation of compound **108** (Hoshino and Sato, 2002) via cation **XXXVII** requires several 1,2 hydride/methyl shifts to generate a C5 carbocation prior to the loss of H6. $C_{30}H_{50}$ compounds **105** and **106** are constituents of the fern genus *Polystichum* and have also been obtained from *A. acidocaldarius* SHC mutants (Pale-Grosdemange et al., 1999; Full and Poralla, 2000; Sato and Hoshino, 2001; Schmitz et al., 2001). Triterpene alcohol **107** is from the fern *Polypodiodes*, which also produces the $C_{30}H_{52}O$ compound **100**. Bicyclic triterpene **108** has been generated by *A. acidocaldarius* SHC mutants (Sato and Hoshino, 2001), but has not been found in nature.

8(26),14(27)-Onoceradiene (**109**) (Arai et al., 1982) is a bis-bicyclic triterpene in which the bicyclic ring structure of **106** was generated from both termini. Compound **109** probably arises by initial cyclization to **106**, which then enters the active site from the other end to form cation**XXXVIII**. Similar deprotonation then yields the symmetrical final product. The pentacycle 14-serratene (**110**) (Ghisalberti et al., 1970; Ageta et al., 1982) is also produced from cation **XXXVIII** but the bis-bicycle undergoes a third cyclization onto the exo-methylene to form the seven-membered ring in cation **XXXIX**. Deprotonation from **XXXIX** generates **110**. Similar to **106**, bicyclic triterpene **107** can also undergo cyclization from the other terminus, and the resulting carbocation is quenched by the 8α-hydroxyl group to produce 8,14-epoxyonocerane (**111**) (Ageta et al., 1982). Compounds **109**–**111** were all found in ferns.

3.3. Tricyclic triterpenes derived from squalene cyclization (Fig. 23)

Squalene (**99**) cyclization leads to the C–C 6-6-5 tricyclic cation, which can have the side chain with either the 13α (**XL**) or 13β (**XLI**) stereochemistry. Direct deprotonation of H27 in cation **XL** or **XLI** produces 13α-14(27),17,21-malabaricatriene (**112**) (Masuda et al., 1989b) or 13β-14(27),17,21-malabaricatriene (**113**) (Masuda et al., 1989b), respectively. Alternatively, cation **XL** or **XLI** can also undergo 1,2 proton/methyl shifts to generate the C8 carbocation **XLII**, which can lose a proton to form 7,17,21-podiodatriene (**114**) (Arai et al., 1989) or its isomer 8,17,21-podiodatriene (**115**) (Arai et al., 1989). Tricyclic squalene products are components of the ferns *Lemmaphyllum* (**112** and **113**) and *Polypodiodes* (**114** and **115**) and can also be generated by *A. acidocaldarius* SHC mutants (Hoshino et al., 1999; Merkofer et al., 1999; Pale-Grosdemange et al., 1999; Hoshino et al., 2000a, 2000b).

3.4. Tetracyclic squalene cyclization products

3.4.1. Tetracyclic 6-6-6-5 triterpenes

Squalene (**99**) can tetracyclize to cations **XLIII** and **XLIV**, the 3-deoxy-analogs of dammarenyl cations **IX**

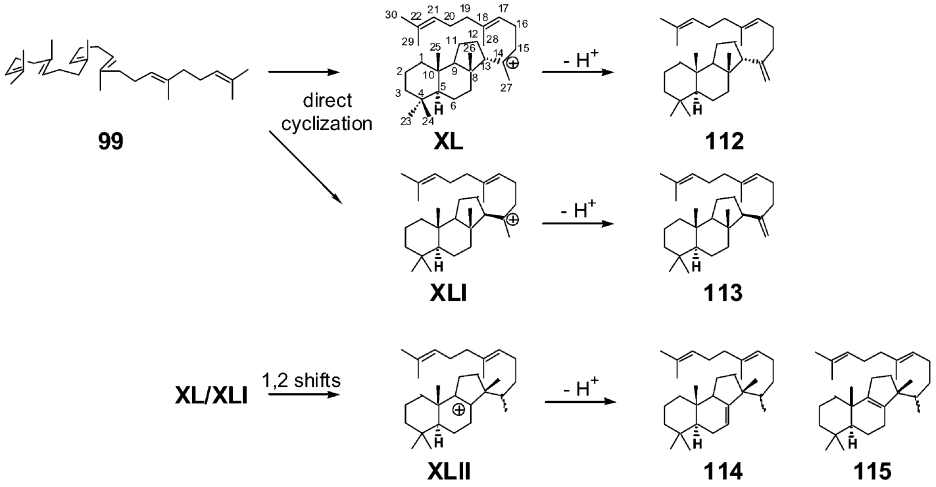

Fig. 23. Tricyclic triterpenes derived from squalene (**99**).

and **X** derived from oxidosqualene (**1**) cyclization (Part I). Immediate deprotonation or hydroxide addition can preserve the stereochemical configuration of C17 in cation **XLIII**/**XLIV**. Dammara-20,24-diene (**116**) (Masuda et al., 1983; Yamashita et al., 1998), 20*S*-dammar-24-en-20-ol (**117**) (Yamashita et al., 1998), and 20*R*-dammar-24-en-20-ol (**118**) (Baker et al., 1976), are derived from intermediate **XLIII**, and in vitro enzymatic products **126**, **127**, and **128** arise from intermediate **XLIV**. Compounds **116** and **117** were isolated from the ferns *Lemmaphyllum* and *Pyrrosia*, respectively, and have also been generated by *A. acidocaldarius* SHC

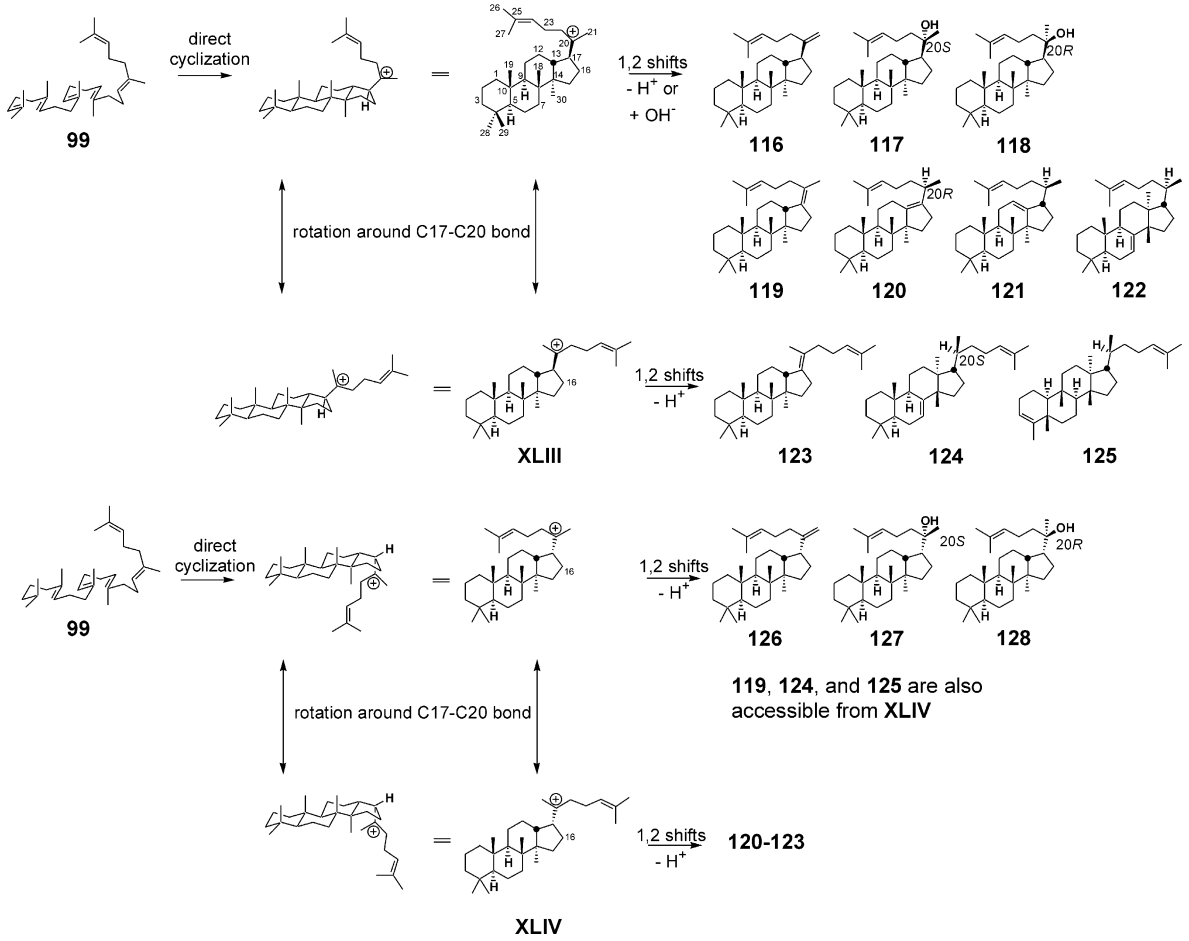

Fig. 24. Tetracyclic 6-6-6-5 triterpenes derived from squalene.

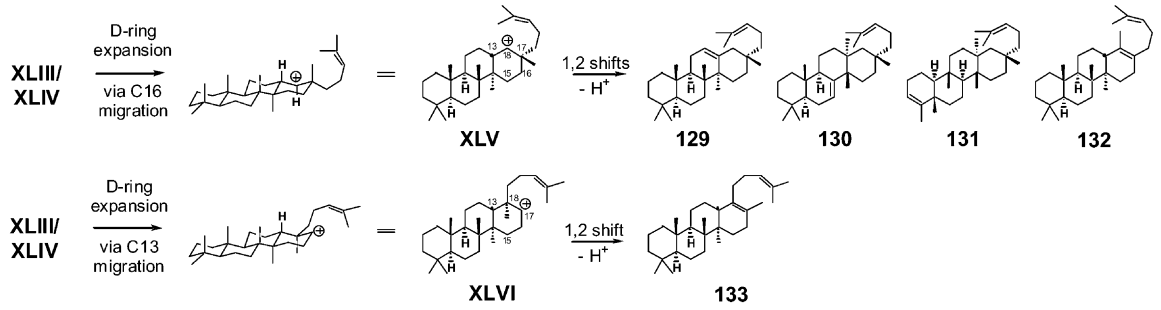

Fig. 25. Tetracyclic 6-6-6-6 triterpenes derived from squalene cyclization.

mutants (Full and Poralla, 2000; Hoshino et al., 2000a). In contrast, **118** and **126–128** are not known from natural sources but have been produced by *A. acidocaldarius* SHC mutants (Hoshino et al., 1999; Merkofer et al., 1999; Full and Poralla, 2000; Hoshino et al., 2000b; Schmitz et al., 2001).

Z-Dammara-17(20),24-diene (**119**) (Toyota et al., 1998) and *E*-dammara-17(20),24-diene (**123**) (Arai et al., 1991) are olefinic isomers that can be derived from intermediate **XLIII**/**XLIV** with opposite side-chain orientations as a result of the rotation around C17–C20 bond. Compound **123** was isolated from the fern *Polypodium*, and **119** was isolated from the moss *Floribundaria* genus. The different origins of the two isomers may imply an evolutionary dichotomy of the corresponding SCs.

Similar to OSC products **23–26**, 20*R*-dammara-13(17),24-diene (**120**) (Arai et al., 1982, 1991), 20*R*-dammara-13,24-diene (**121**) (Hoshino et al., 2000b), and eupha-7,24-diene (**122**) (Arai et al., 1982; Masuda et al., 1983) share the 20*R* stereochemical configuration, which derives either from 17α-H migration in cation intermediate **XLIII** with the side chain anti to C16 or from 17β-H migration in **XLIV** with the side chain *syn* to C16 (Fig. 24). Conversely, tirucalla-7,24-diene (**124**) (Arai et al., 1982; Masuda et al., 1983) and aonena-3,24-diene (**125**) (Arai et al., 1989) have the 20*S* stereochemical configuration, originating from 17α-H migration in cation intermediate **XLIII** with the side chain *syn* to C16 or from 17β-H migration in **XLIV** with the side chain anti to C16 (Fig. 24). Compounds **120**, **122**, **124**, and **125** were isolated from ferns, and **121** was produced by native (Pale-Grosdemange et al., 1998) and mutant *A. acidocaldarius* SHC (Full and Poralla, 2000; Hoshino et al., 2000b) and has not been found in nature. Natural triterpenes **120** and **122** can also be generated by *A. acidocaldarius* SHC mutants (Full and Poralla, 2000; Hoshino et al., 2000a, 2000b).

3.4.2. Tetracyclic 6-6-6-6 triterpenes derived from D ring expansion through C16 or C13 migration

Three tetracyclic 6-6-6-6 $C_{30}H_{50}$ natural products, 12,21-baccharadiene (**129**) (Masuda et al., 1983), 7,21-lemmaphylladiene (**130**) (Masuda et al., 1983), and 3,21-shionadiene (**131**) (Masuda et al., 1983), were isolated from the fern genus *Lemmaphyllum*. Compounds **132** and **133** have not been found in nature but have been generated by an *A. acidocaldarius* SHC mutant (Hoshino et al., 2000a). The formation of **129–132** starts with D-ring expansion (C16 migration) in cation intermediate **XLIII**/**XLIV** to form cation **XLV**, followed by varied 1,2 shifts and deprotonations. Compound **133**, however, results from C13 migration/D-ring expansion of cation **XLIII**/**XLIV** to **XLVI** with subsequent methyl shift and deprotonation (Fig. 25).

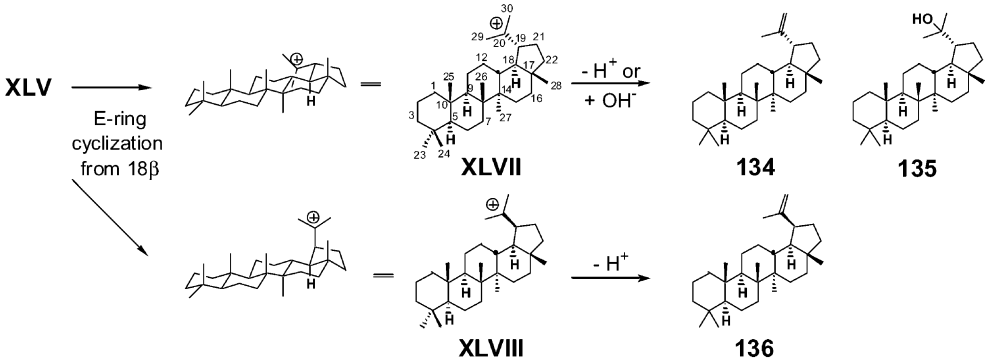

Fig. 26. Lupane 6-6-6-6-5 triterpenes derived from squalene (D-ring expansion via C16).

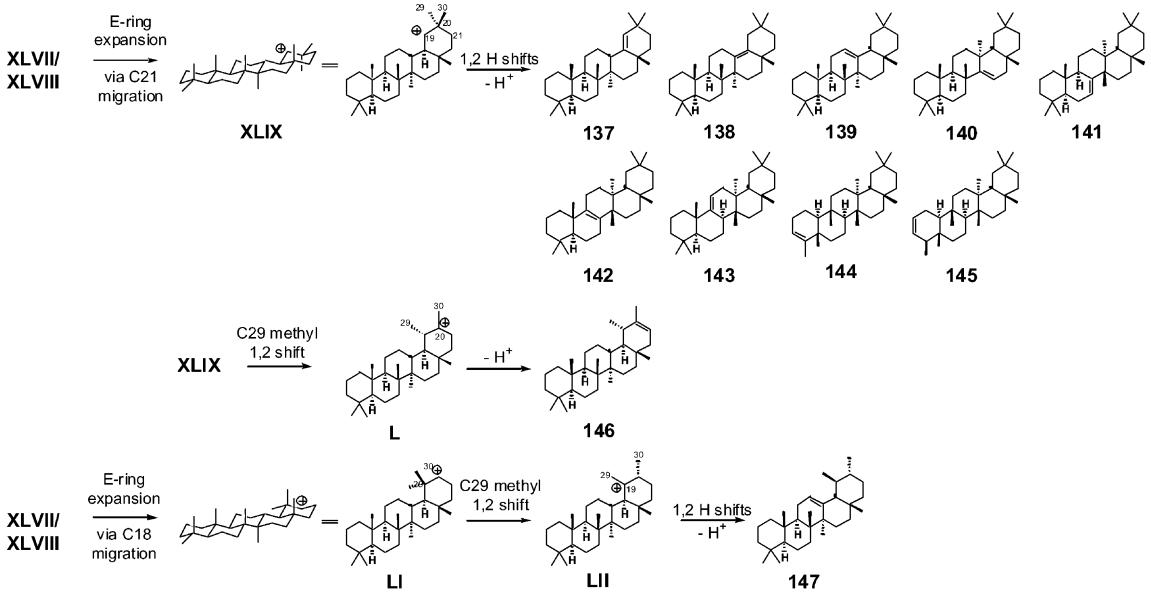

Fig. 27. Pentacyclic 6-6-6-6-6 triterpenes derived from deoxylupyl cations.

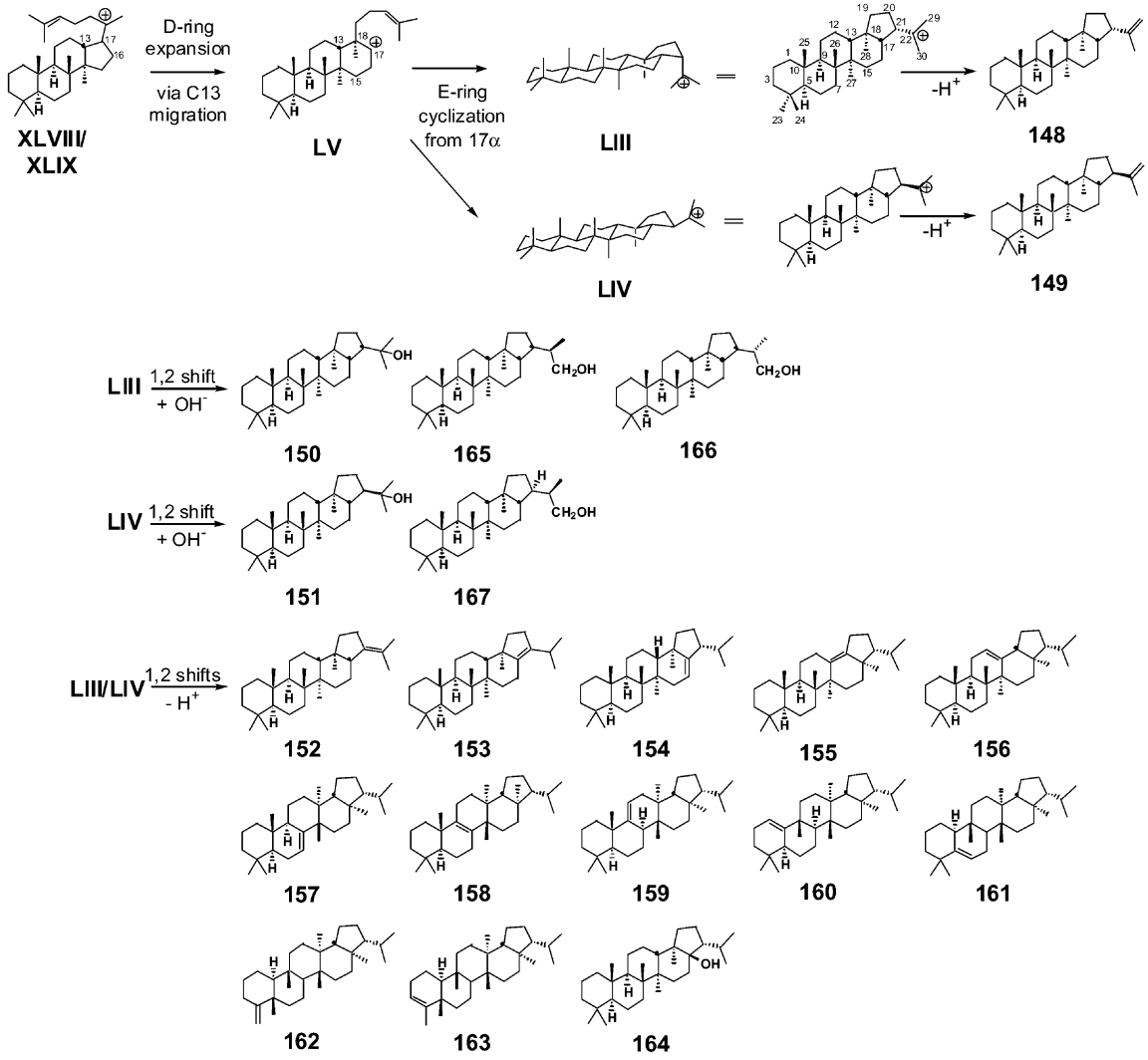

Fig. 28. Hopane 6-6-6-6-5 triterpenes derived from squalene cyclization.

3.5. Pentacyclic squalene cyclization products

3.5.1. Lupane 6-6-6-6-5 triterpenes

Lupyl cation **XLVII** and its epimer **XLVIII** are generated from cation **XLV** via C16 migration/D-ring expansion. Deprotonation of H30 from **XLVII** and **XLVIII** leads to 20(29)-lupene (**134**) and 19β-20(29)-lupene (**135**) (Wenkert et al., 1978; Ageta et al., 1982). Alternatively, cationic intermediate **XLVII** can be quenched by water to form 20-lupanol (**136**) (Hirohara et al., 2000). Compounds **134**–**136** were all isolated from ferns, with **134** and **135** from *Lemmaphyllum*, and **136** from *Polypodiodes* (Fig. 26).

3.5.2. Pentacyclic 6-6-6-6-6 triterpenes derived from the lupyl cation by ring expansion

To form the 6-6-6-6-6 ring system, cationic intermediates **XLVII**/**XLVIII** can undergo E-ring expansion via C21 migration to yield germanicyl cation **XLIX**. Cation **XLIX** is the precursor of a variety of natural products, including 18-oleanene (**137**) (Ageta and Arai, 1983; Ageta et al., 1995), 13(18)-oleanene (**138**) (Ageta et al., 1995), 12-oleanene (**139**) (Ageta and Arai, 1983; Ageta et al., 1995), 14-taraxerene (**140**) (Ageta and Arai, 1983; Ageta et al., 1995; Bates et al., 1998), 7-multiflorene (**141**) (Ageta and Arai, 1983; Ageta et al., 1995), 8-multiflorene (**142**) (Ageta and Arai, 1983), 9(11)-multiflorene (**143**) (Ageta and Arai, 1983; Ageta et al., 1995), 3-friedelene (**144**) (Ribas-Marques and Fernandez-Salgado, 1974; Hui and Li, 1976; Ageta and Arai, 1983; Ageta et al., 1995), and 2-friedelene (**145**) (Ribas-Marques and Fernandez-Salgado, 1974). The formation of 20-taraxastene (**146**) (Ageta and Arai, 1983) is also rationalized from cation **XLIX** after 29α-methyl shift to yield the taraxastane type cation **L**, which is neutralized by deprotonation. Alternatively, cation **XLIX** can rearrange via C18 migration to **LI** followed by 29α-methyl migration to yield 18α-ursyl cation intermediate **LII**. The only known example derived from cation **LII** is 12-ursene (**147**, 3-deoxy α-amyrin) (Siddiqui et al., 1988).

Although almost all mono-, bi-, tri-, and tetracyclic natural triterpenes cyclized from squalene (**99**) are fern constituents (except **119** from moss), pentacyclic 6-6-6-6-6 $C_{30}H_{50}$ natural compounds arise from very different origins. Triterpenes **137**, **139**–**144**, and **146** were found in the well-studied fern *Polypodium*. However, **139** was also isolated from angiosperm plants, the genus of *Achillea* and *Erysimum*, **140** from *Ascomycota cladonia*, and the 3α,4α-epoxide of **141** from the angiosperm plant *Castanopsis*. In addition, **138** is present as geological deposit in shale oil, possibly as a component of some ancient organism, and **145** and **147** are from angiosperm plants, cork oak and rose-bay, respectively (Fig. 27).

3.5.3. Hopane 6-6-6-6-5 triterpenes derived from squalene (Fig. 28)

Hopanoids are the most extensively studied squalene cyclization products because of their important physiological role in prokaryotes as well as their prevalence in lower plants. The hopyl cationic intermediates **LIII** and **LIV** can be viewed as the derivatives of cation **XLIV** through D-ring expansion (C13 migration) to **LV** followed by E-ring cyclization. Alternatively, cations **LIII**/**LIV** can be considered as directly cyclized from squalene (**99**). Whether the 6-6-6-6-5 ring cyclization is concerted is still under debate.

Cations **LIII** and **LIV** can be quenched by immediate deprotonation, leading to 22(29)-hopene (**148**) (Shiojima and Ageta, 1990; Ageta et al., 1993; Arai et al., 1994) and diploptene (**149**, hopene B) (Ageta et al., 1963, 1993; Ageta and Shiojima, 1968; Wilkins et al., 1987) respectively. The C22 carbocation in **LIII** and **LIV** can also be directly quenched by the addition of water, yielding 21*S*-22-hopanol (**150**, diplopterol, hydroxyhopane) (Baddeley et al., 1961; Ageta et al., 1963, 1993; Kamaya et al., 1990; Grammes et al., 1994) and 21*R*-22-hopanol (**151**) (Arai et al., 1991). 21-Hopene (**152**, hopene A) (Marsili et al., 1971; Wilkins et al., 1987; Shiojima and Ageta, 1990; Ageta et al., 1994) can be generated from either **LIII** or **LIV** by loss of H21.

Cationic intermediates **LIII**/**LIV** can also undergo various hydride and methyl shifts prior to deprotonation or hydroxylation, contributing to the diversity of hopanoid natural products including 17(21)-hopene (**153**, hopene I) (Bottari et al., 1972; Shiojima and Ageta, 1990; Ageta et al., 1994), 16-hopene (**154**) (Shiojima and Ageta, 1990; Ageta et al., 1994), 13(18)-neohopene (**155**, hopene II) (Ageta and Shiojima, 1968; Bottari et al., 1972; Ageta et al., 1987, 1994), 12-neohopene (**156**) (Ageta and Shiojima, 1968; Wu et al., 1982; Ageta et al., 1987, 1994; Shiojima and Ageta, 1990), (**157**) 7-fernene (Ageta et al., 1964, 1994; Ageta and Iwata, 1966; Masuda et al., 1989a), 8-fernene (**158**, isofernene) (Ageta et al., 1963, 1994; Ageta and Iwata,

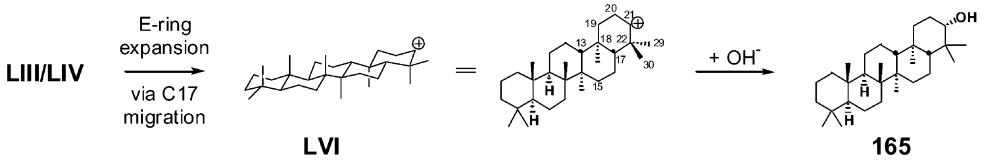

Fig. 29. Tetrahymanol is generated from the hopyl cation via E-ring expansion.

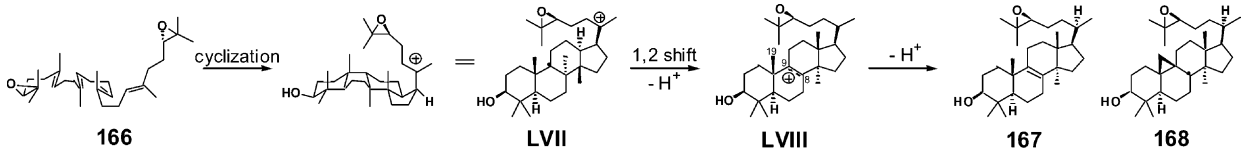

Fig. 30. 2,3-(*S*)-22,23-(*S*)-bis-Oxidosqualene (**166**) is cyclized to the 24,25-epoxyprotosteryl cation **LVII** en route to **167** and **168**.

1966; Masuda et al., 1989a), 9(11)-fernene (**159**, davallene, fernene) (Ageta et al., 1963, 1994; Ageta and Iwata, 1966; Ghisalberti et al., 1970; Wollenweber et al., 1981; Masuda et al., 1989a), 1(10)-adianene (**160**) (Shiojima and Ageta, 1990), 5-adianene (**161**) (Ageta et al., 1964, 1994; Masuda et al., 1989a), 4(23)-filicene (**162**) (Hirohara et al., 1997), 3-filicene (**163**, filicene) (Ageta et al., 1964, 1993, 1994; Ageta and Iwata, 1966), and 17β-hopanol (**164**) (Ageta et al., 1987). The usual hydride shift transforming the tertiary C22 carbocation in **LIII**/**LIV** to the primary C29 carbocation is required to yield 22*R*-29-hopanol (**165**, neriifoliol) (Horvath et al., 1975; Ageta et al., 1993), 22*S*-29-hopanol (**166**, dryocrassol) (Ageta et al., 1975, 1993), and 21*R*,22ϵ-29-hopanol (**167**) (Goswami et al., 1979; Ageta et al., 1993), which are formed by the addition of a hydroxide equivalent at C29.

Hopanoids **148–67** have diverse ecological origins, and some of them have important biological functions. Diplopterol (**150**) is commonly produced by bacteria, triterpenes **149** and **153** were discovered in geological sediments, and **149** and **152** were isolated from mosses. Compounds **149**, **155–59** and **163** are relatively widespread fern components, and **152–56** have been produced by *A. acidocaldarius* SHC mutants (Hoshino et al., 2000a, b).

3.5.4. Pentacyclic 6-6-6-6-6 triterpenes derived from the hopyl cation

Cationic intermediate **LIII**/**LIV** can undergo further E-ring expansion to the secondary carbocation **LVI**. Addition of a hydroxide equivalent yields 3-gammaceranol (**165**, tetrahymanol) (Mallory et al., 1963; Shiojima et al., 1989a), a constituent of the ciliate *Tetrahymena pyriformis* (kingdom Protoctista) and many ferns (Fig. 29).

4. Bis-oxidosqualene cyclization

Several triterpenoids contain oxidation that is structurally consistent with arising from a bis-oxidosqualene (**166**) substrate. Two general modes of bis-oxidosqualene (**166**) cyclization have been established experimentally. Cyclization and rearrangement reactions can yield epoxy derivatives of known oxidosqualene cyclase product. Other natural products arise when the resultant epoxyalcohol retains the 18,19 olefin and a second cyclization is initiated from the distal 22,23-epoxide. Finally, some natural triterpenoids with 5- or 6-membered oxetane rings are structurally consistent with a single cyclization in which the terminal epoxide undergoes electrophilic attack by a carbocation generated in the initial cyclization.

The best studied example of bis-oxidosqualene (**166**) cyclization is the conversion of 2,3-(*S*)-22,23-(*S*)-bis-oxidosqualene (**166**) to 24.25-epoxylanostan-3-ol (**167**, Fig. 30) (Field and Holmlund, 1977). Squalene (**99**) is oxidized by squalene epoxidase to oxidosqualene, and in conditions when lanosterol synthase is limiting, the distal terminus of oxidosqualene (**1**) can re-enter the squalene epoxidase and be epoxidized. The resultant 2,3-(*S*)-22,23-(*S*)-bis-oxidosqualene (**166**) is a substrate for the native lanosterol synthase and undergoes the conventional cyclization, generating the epoxylanosterol (**12**, Fig. 4). Epoxylanosterol (**1**) accumulation appears to be a key negative feedback signal for maintaining sterol homeostasis in yeast and mammals (Spencer, 1994;

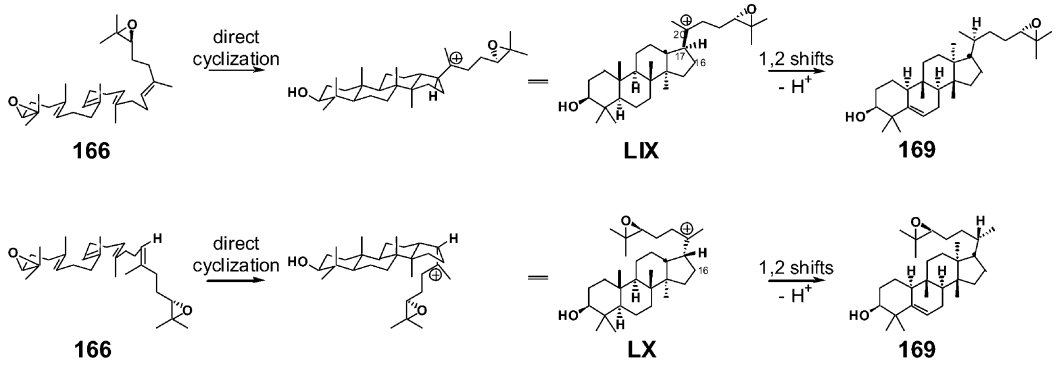

Fig. 31. 2,3-(*S*)-22,23-(*S*)-bis-Oxidosqualene (**166**) cyclization to reissantenol oxide through a dammarenyl epoxide cation.

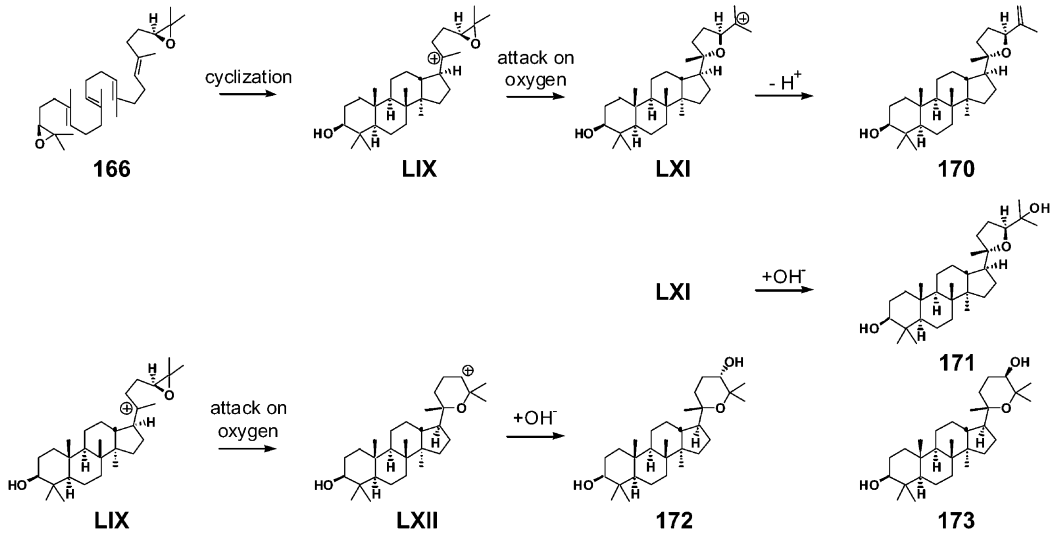

Fig. 32. Tetrahydrofuran and tetrahydropyran triterpene alcohols and diols that could be derived from 2,3, 22,23-bis-oxidosqualene cyclization.

Gardner et al., 2001). Similarly, 24,25-(*S*)-epoxycycloartan-3-ol (**168**) (De Pascual Teresa et al., 1987; Della Greca et al., 1994) is the epoxy derivative of the corresponding plant sterol precursor cycloartenol (**16**). Epoxide **168** arises from the normal action of cycloartenol synthase on 2,3-(*S*)-22,23-(*S*)-bis-oxidosqualene (Heintz et al., 1970).

Reissantenol oxide (**169**) (Gamlath et al., 1989) is the 24,25-(*S*)-epoxide of the highly rearranged dammarane euferol (**31**). The terminal olefin of oxidosqualene (**1**) does not participate in cyclization to euferol (**31**). Provided that the enzyme lacks strict substrate specificity, euferol synthase should cyclize 2,3-(*S*)-22,23-(*S*)-bis-oxidosqualene (**166**) to reissanteol oxide (**169**). However, this system has not been experimentally examined. Both the 17α- epimer **X** and the 17β- epimer **IX** of the dammarenyl cation are plausible intermediates en route to euferol (**31**) (Fig. 5). Cyclization of bis-oxidosqualene (**166**) to the 17β-epoxydammarenyl cation **LIX** followed by the 17α-hydride shift with the side chain *syn* to C16 would generate the 20*S* stereocenter. Extensive 1,2 shifts and deprotonation from C6 would yield **169**. Alternatively, **169** could arise from initial cyclization to the 17α epimer **LX** and subsequent 17β-hydride shift with the side chain *anti* to C16, followed by rearrangement and deprotonation as above (Fig. 31).

An experimentally unexplored but mechanistically reasonable mode of bis-oxidosqualene cyclization to yield tetrahydrofuran or tetrahydropyran natural products involves the distal epoxy group in cyclization. One readily rationalized structure is the 3β,20*S*,24*S*-epoxy-25-dammaren-3-ol richenol (**170**) (Aalbersberg and Singh, 1991). The 3β-hydroxyl in richenol betrays its origin from a 2,3(*S*)-oxidosqualene derivative, and because the dammarenyl ring system has not undergone rearrangement, the 17β stereochemistry mandates the 17β intermediate carbocation **LIX**. The bis-oxidosqualene precursor should have 3*S*,22*S* stereochemistry because the 24*S* stereocenter in richenol is preserved from C22 in the bis-epoxide substrate, and the intermediate cation consequently should have the structure of **LXI**. The 20*S* stereochemistry would be established by epoxide attack from the pro-*S* face of the planar C17 carbocation. If **LXI** did not undergo deprotonation but instead were quenched by a hydroxide equivalent, the 3β,20*S*,24*S* tetrahydrofuran diol 3β-cabraleadiol (**171**) (De Pascual Teresa et al., 1979) would result. Alternatively, **171** could arise by electrophilic attack on a diol as in **174** below. Compound **170** could form similarly by electrophilic addition on an allylic alcohol or by dehydration of **171**.

3β-Acetyl-20,25-epoxydammarane-24α-ol and 3β-acetyl-20,25-epoxydammarane-24β-ol are acetylated tetrahydropyran triterpene diols from *Forsythia suspense* (Rouf et al., 2001). These compounds are presumably acetylated derivatives of 20*S*,25-epoxydammarane-3β,24*S*-diol (**172**) and 20*S*,25-epoxydammarane-3β,24*R*-diol (**173**). 17β-Epoxydammarenane cation **LIX** could attack the epoxide to generate the C24 cation and thereby generate **LXII**. Addition of a hydroxide equivalent could generate **172** and **173**. A variety of known tetrahydrofuran and tetrahydropyran natural products are isomeric to **170–173** but are epimeric C3, C20, and C24. The potential origins of these compounds are less obvious and remain to be investigated.

Malabaricanediol **174** (Sobti and Dev, 1968) is a triterpene diol with a tetrahydrofuran ring system that resembles that in the dammarane derivative **171**. However, **174** has one fewer carbocyclic ring than **171**, the distal olefin remains unmodified, and the tetrahydrofuran ring is positioned one prenyl group closer to the proximal terminus. Malabaricanediol (**174**) has been

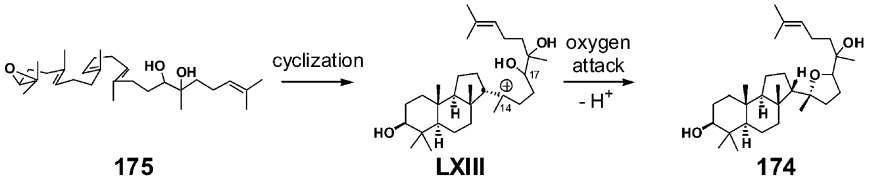

Fig. 33. Malabaricanediol (**174**) is structurally consistent with cyclization from 2,3-epoxy-18,19-dihydrosqualene-18,19-diol (**175**).

semisynthesized from 2,3-epoxy-18,19-dihydrosqualene-18,19-diol (**175**) via carbocation **LXIII** as shown in Fig. 33, and the biosynthesis was proposed to proceed similarly (Sharpless, 1970). Cyclization of a bis-epoxide substrate as outlined above for **171** is a plausible alternative (Fig. 33).

bis-Oxidosqualene cyclization products that have fewer than four carbocyclic rings retain one or more olefins that can in principle undergo a second cyclization initiated by protonation of the distal epoxide. α-Onocerin (**176**) (Barton and Overton, 1955) is a product of 2,3-(*S*)-22,23-(*S*)-bis-oxidosqualene (**166**) that arises by separate bicyclization from each terminus. The biosynthesis of this bis-bicyclic triterpene diol from bis-oxidosqualene (**166**) has been established in a cell-free system from *Ononis spinosa* (Rowan et al., 1971). Protonating one epoxide initiates bicyclization to cation **LXIII**, and proton abstraction from C26 yields preonocerin (**177**) (Rowan and Dean, 1972). Protonating the distal epoxide initiates a second bicyclization to cation **LXIV**, and deprotonation as before generates onocerin.

13-Serratene-3β,21α-diol (**178**) (Inubushi et al., 1964) is a pentacyclic triterpene diol that contains an unusual seven-membered C-ring. Serratenediol (**178**) could arise by a route similar to that established for onocerin (**176**); during the cyclization cascade from preonocerin (**177**), the carbocation in **LXIV** can attack the B-ring exo-methylene to form carbocation **LXV**, which can then eliminate a proton from C-9 to form the tetrasubstituted olefin (Fig. 32). Various spruce species contain other natural 13-serratene-3,21-diols in which either or both alcohols have inverted stereochemistry. These compounds are probably metabolites of 13-serratene-3β,21α-diol in which either or both equatorial alcohols was oxidized to a ketone and reduced by hydride delivery from the β-face to generate the axial alcohol. A similar process is believed to generate diverse conifer 3α-hydroxytriterpenoids, in which the stereochemistry at C3 is inverted from that in 3(*S*)-oxidosqualene (Fig. 34).

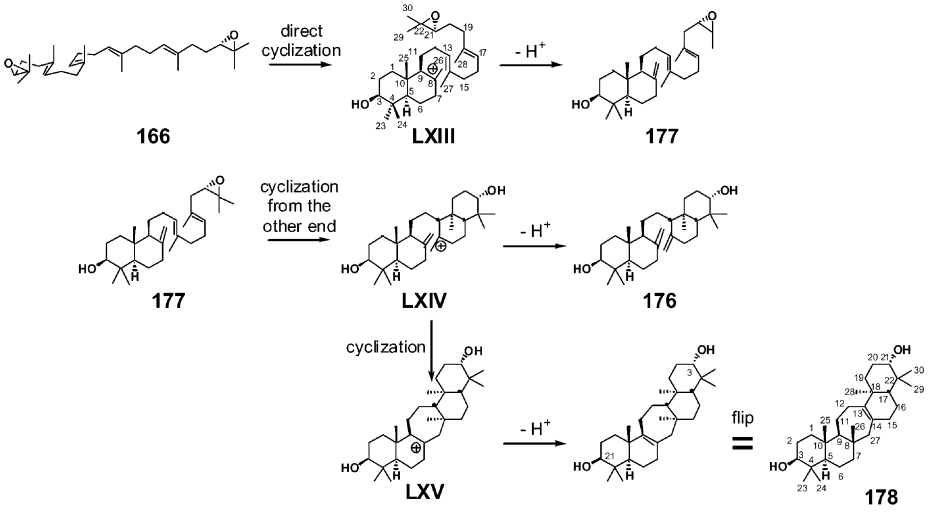

Fig. 34. Bis-oxidosqualene (**166**) cyclizes from both terminal epoxides to generate preonocerin (**177**), onocerin (**176**), and serratenediol (**178**).

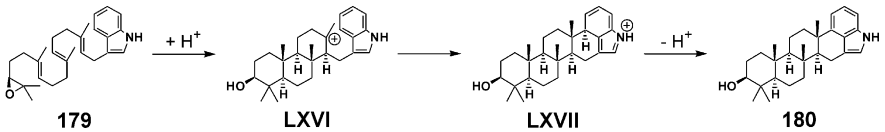

Fig. 35. Lupeol synthase can convert epoxygeranylgeranyl indole (**179**) to the fungal indole diterpenoid petromindole (**180**).

5. Future prospects

In the half a century since it was proposed, myriad products of squalene (**99**), oxidosqualene (**1**), and bis-oxidosqualene (**166**) cyclization have been discovered that are structurally consistent with the biogenetic isoprene rule. Numerous enzymological experiments have been carried out that confirmed and developed this theory. During the past decade, the biosyntheses of some of the most important triterpene alcohols have been further established using cloned and heterologously expressed enzymes. These recombinant approaches offer powerful new tools with which to uncover new triterpenoids; in the past few years, more new cyclization products have been uncovered from modified enzymes than by classical isolation during the same period. Experiments with recombinant enzymes have recently established that these enzymes can generate natural products that are not triterpenoids. For example, an *Arabidopsis* cyclase that normally converts oxidosqualene to pentacyclic triterpene alcohols and diols can cyclize epoxygeranylgeranyl indole (**179**) through the cationic intermediates **LXVI** and **LXVII** to the indole diterpene known as petromindole (**180**, Fig. 35) (Xiong et al., 2003). The substrate specificity of these enzymes remains incompletely defined, and considerable opportunity exists to uncover additional squalene cyclase and oxidosqualene cyclase products.

Note added in proof

Three compounds generated by recombinant and mutant enzymes were not listed in the Dictionary of Natural Products and they eluded our initial literature search. A *Panax ginseng* β-amyrin synthase mutant in which Tyr261 was mutated to His generated two novel tetracyclic isomers: the 20(22) *Z* (**181**) and *E* (**182**) isomers of dammara-20(22),24-dien-3β-ol (numbered as in Fig. 6) (Kushiro et al., 2000b). The 17β stereochemistry of these unrearranged dammaranes establishes that they arise from cation **IX** (Fig. 36).

The *Arabidopsis thaliana* lupeol synthase generates significant amounts of 3,20-dihydroxylupane (**183**) by adding a hydroxide equivalent to lupyl cation (**XVI**) (Fig. 37) (Segura et al., 2000; Kushiro et al., 2000b).

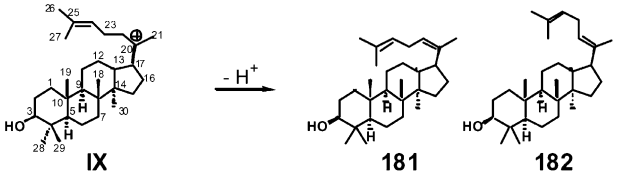

Fig. 36. A mutant synthase β-amyrin synthase can abstract a proton from dammarenyl cation (**IX**) to form the olefinic dammaradienol isomers (**181** and **182**).

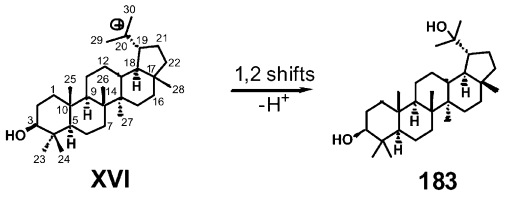

Fig. 37. Dihydroxylupane (**183**) arises from hydrolytic quenching of lupyl cation (**XVI**).

Including compounds **181** ansd **182** with those described above brings to 100 the number of known compounds that appear to be oxidosqualene cyclization products

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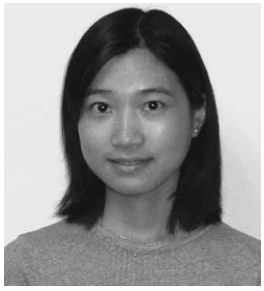

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