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Origin of Structural Diversity in Natural Triterpenes: Direct Synthesis of *seco*-Triterpene Skeletons by Oxidosqualene Cyclase

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Abstract: At1g78500, one of the oxidosqualene cyclase (OSC) homologues from *Arabidopsis thaliana*, was expressed in a lanosterol synthase-deficient yeast strain and the products were analyzed. In addition to the known triterpenes, this OSC was found to produce two new triterpenes, the structures of which were determined by NMR and MS analyses. The new triterpenes are C-ring-*seco*-β-amyrin (**1**) and C-ring-*seco*-α-amyrin (**2**) and named β-*seco*-amyrin and α-*seco*-amyrin, respectively. β-*seco*-Amyrin is produced from the oleanyl cation through bond cleavage between C8 and C14, and α-*seco*-amyrin is produced from the ursanyl cation in the same manner. Together with Grob fragmentation catalyzed by another OSC (marneral synthase) from *A. thaliana*, the formation of *seco*-amyrins by this OSC revealed that OSCs not only catalyze carbon−carbon bond formations and Wagner−Meerwein rearrangements but also cleave preformed ring systems in cationic intermediates. Based on this information, direct production of other natural *seco*-triterpenes by OSCs is proposed.

Introduction

A characteristic feature of natural products is their structural diversity, which is generated by a sequence of enzymatic reactions in the producing organisms. The primary origin of structural diversity arises in skeletal formation catalyzed by biosynthetic enzymes that utilize various chemistries including carbon−carbon bond formation by aldol and Claisen condensations, electrophilic addition of carbocation to alkenes, etc. These carbon−carbon bond formations serve as the key reactions to synthesize a variety of complex organic molecules in nature. Not only in the biosynthesis of natural products, these reactions are also key reactions in synthetic chemistry.

The terpenoids form a large and structurally the most diverse group of natural products derived from C5 isoprene units. Terpene skeletons are produced from acyclic substrates by carbocation chemistry through successive electrophilic additions followed by rearrangements. Triterpene biosynthesis has some features distinct from those of lower terpenes.[1] In higher plants, triterpene synthesis usually starts by protonation of an epoxide at one end of the substrate oxidosqualene (**3**) to produce carbocation, while mono-, sesqui-, and diterpene syntheses, with some exceptions like those involved in gibberellin biosynthesis, are initiated by the elimination of a diphosphate group. Lower terpene synthases often yield macrocyclic rings, while triterpene synthases without exception produce fused ring systems by successive ring formation. As a result, fused multicyclic triterpenes have a number of chiral centers. The reaction catalyzed by triterpene synthase is thus regarded as one of the most complex reactions occurring in nature.

More than 100 triterpene skeletons have been reported in nature.[2] For over a half-century since the historical proposal of the biogenetic isoprene rule by the ETH group,[3] the fascinating mechanism of triterpene formation has attracted considerable attention from leading scientists.[1] The introduction of enzymology and molecular biology, in particular, in this field has provided an opportunity to study reaction mechanisms at enzymatic levels. So far, more than 30 oxidosqualene cyclases (OSCs) have been cloned and characterized.[4] β-Amyrin synthase[5] and lupeol synthase[6] were the first triterpene synthases to be cloned. Mutagenesis studies on these enzymes showed that only one amino acid substitution could dramatically alter their product specificity. Rational interconversion between

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(1) Abe, I.; Rohmer, M.; Prestwich, G. D. *Chem. Rev.* **1993**, *93*, 2189−2206.
(2) Xu, R.; Fazio, G. C.; Matsuda, S. P. T. *Phytochemistry* **2004**, *65*, 261−291.
(3) (a) Eschenmoser, A.; Ruzicka L; Jeger, O.; Arigoni, D. *Helv. Chim. Acta* **1955**, *38*, 1890−1940. (b) Eschenmoser, A.; Arigoni, D. *Helv. Chim. Acta* **2005**, *88*, 3011−3050.
(4) Phillips, D. R.; Rasbery, J. M.; Bartel, B.; Matsuda, S. P. T. *Curr. Opin. Plant Biol.* **2006**, *9*, 305−314.
(5) (a) Kushiro, T.; Shibuya, M.; Ebizuka, Y. *Eur. J. Biochem.* **1998**, *256*, 238−244. (b) Kushiro, T.; Shibuya, M.; Ebizuka, Y. *Towards Natural Medicine Research in the 21st Century*, *Excerpta Medica International Congress Series*; Ageta, H., Aimi, N., Ebizuka, Y., Fujita, T., Honda, G., Eds; Elsevier Science: Amsterdam, 1998; pp 421−427.
(6) (a) Herrera, J. B. R.; Bartel, B.; Wilson, W. K.; Matsuda, S. P. T. *Phytochemistry* **1998**, *49*, 1905−1911. (b) Shibuya, M.; Zhang, H. Endo, A.; Shishikura, K.; Kushiro, T.; Ebizuka, Y. *Eur. J. Biochem.* **1999**, *266*, 302−307.

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β-amyrin synthase and lupeol synthase has been achieved.[7] Extensive cloning studies have revealed the presence of OSCs with different product specificity. They are isomultiflorenol synthase,[8] cucurbitadienol synthase,[9] thalianol synthase,[10] marneral synthase,[11] and arabidiol synthase.[12] Furthermore, in addition to these monofunctional synthases yielding one specific product, multifunctional synthases producing more than one product have been cloned. These include a mixed amyrin synthase from *Pisum sativum*,[13] a multifunctional lupeol synthase (LUP1),[6a] another multifunctional OSC yielding more than nine products from *Arabidopsis thaliana*,[14] etc. These results suggest that more than 100 triterpene skeletons reported from nature are elaborated by contribution of both mono- and multifunctional triterpene synthases. So far, the enzymes responsible for the formation of more than 20 triterpene skeletons have been identified, leaving the producing enzymes of the majority of the other triterpene skeletons unidentified. The identification and characterization of such enzymes are highly expected to uncover the origin of diverse triterpene skeletons in nature.

We have been engaged in the functional analysis of triterpene synthase homologues of *A. thaliana*, a model in the field of plant science. We found that one of them can catalyze C-ring cleavage of pentacyclic triterpene intermediates and yield novel *seco*-triterpenes. Based on these findings, we propose here that some of the naturally occurring *seco*-triterpenes could be directly produced by OSCs.

Experimental Section

Cloning of At1g78500. Total RNA of *A. thaliana* was prepared from 3- to 4-week-old seedlings (ca. 5 g) using the phenol-SDS method and lithium chloride precipitation.[5a] The total RNA obtained was subjected to RT-PCR to give the first strand cDNA as described in the literature.[5a] The full-length cDNA was obtained using nested PCR. The first PCR was carried out in a 100 μL solution containing primers (5′-TTCTAAAAGGTTTGATACAAA-3′ and 5′-TAAGCTTTGGATGCGAAGTCT-3′, 1 μg each), the first strand cDNA (3 μL), dNTP mixture (0.2 mM), 10× buffer (10 μL), and *Ex-Taq* DNA polymerase (2.5 U, TAKARA BIO INC.). PCR was carried out for 30 cycles using Robocycler Gradient 40 (Stratagene) with the program 94 °C for 1 min, 58 °C for 2 min, 72 °C for 3 min, and final extension at 72 °C for 10 min. After PCR amplification, the product was applied to a Suprec 02 filter (TAKARA BIO INC.) to remove the primers. Using the first PCR product as the template, the second PCR was carried out in the same manner as the first PCR except for the primers used (5′-TTGA**GGTACC**ATGTGGAGGCTGAAGATCGGG-3′ and 5′-GATGCG**GAATTC**TCAACAGAGGGGCACGCGCAG-3′) and yielded a 2.3-kb DNA fragment corresponding to the full length. The fragment was separated on agarose gel electrophoresis, purified using a Wizard PCR Preps Kit (Promega), digested with *Kpn*I and *Eco*RI, and then subcloned into the *Kpn*I and *Eco*RI sites of yeast expression vector pYES2 (Invitrogen). The full length cDNA clone obtained was sequenced in both strands. This sequence is available under accession number AB274959 in the DDBJ sequence database.

Functional Expression of At1g78500 in Yeast GIL77 and Isolation of Products. The full length At1g78500 clone obtained was transferred into a *Saccharomyces cerevisiae* strain GIL77 (*gal2 hem3-6 erg7 ura3-167*).[5a] GIL77 harboring At1g78500 was grown at 30 °C in synthetic complete medium (6 L) lacking uracil (SC-U) with 2% glucose as a carbon source and supplemented with ergosterol (20 μg/mL), hemin chloride (13 μg/mL), and Tween 80 (5 μg/mL). After 2 days, the cells were collected by centrifugation, resuspended in SC-U (6 L) without glucose, supplemented with hemin chloride, ergosterol, Tween 80, and 2% galactose, and incubated at 30 °C for 1 day. Cells were collected and resuspended in 0.1 M potassium phosphate (6 L), pH 7.0, supplemented with 3% glucose and hemin chloride, and further incubated for 1 day at 30 °C. Cells were finally collected and disrupted with 20% KOH/50% EtOH. The cyclization products were extracted with hexane to give the crude extract (348 mg), which was subjected to silica gel chromatography (Wako gel C-300, 2.4 cm × 48 cm) eluted with hexane/ethyl acetate (9:1, 20 mL/fraction). Fractions 15 and 16 were combined together (6.8 mg) and further separated with HPLC (YMC-pack ODS-A302, 4.6 mm × 250 mm, 60% acetonitrile, 1.2 mL/min, 32 °C, UV abs 202 nm) to give compounds **1** (2.7 mg) and **2** (1.5 mg). Their ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) data are shown in Table 1.

Compound 1. $[\alpha]_D^{19}$: +12.0° (c = 0.10 in $CHCl_3$); HREIMS (positive): m/z 426.3853, calcd for $C_{30}H_{52}O_2$ 426.3862.

Compound 2. $[\alpha]_D^{19}$: +10.1° (c = 0.07 in $CHCl_3$); HREIMS (positive): m/z 426.3847, calcd for $C_{30}H_{52}O_3$ 426.3862.

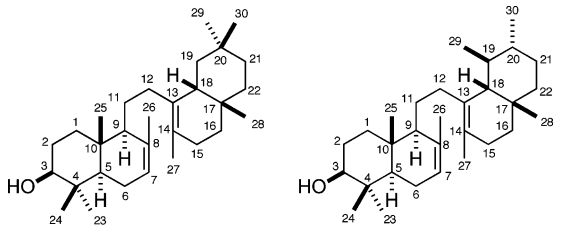
Table 1. NMR Data of **1** and **2**

	1		2	
no.	^{13}C	^{1}H	^{13}C	^{1}H
1	37.1	1.92(α), 1.19(β)	37.2	1.89 (*dt*, 9.6, 3.7 Hz), 1.17
2	27.5	1.55−1.70	27.5	1.67, 1.60
3	79.2	3.25 (*dd*, 11.1, 4.8 Hz)	79.2	3.25 (*dd*, 11.6, 4.3 Hz)
4	38.6		38.6	
5	49.7	1.20	49.7	1.19
6	23.5	1.97	23.5	1.96
7	121.8	5.40 (1H, brs)	121.7	5.39 (1H, brs)
8	135.4		135.5	
9	55.7	1.58	55.7	1.54
10	36.9		37.0	
11	26.0	1.42, 1.14	25.3	1.41, 1.04
12	34.9	2.41 (*dt*, 12.8, 5.7 Hz), 1.58	39.3	2.69 (*dt*, 13.3, 5.7 Hz), 1.52
13	134.2		134.5	
14	123.6		124.8	
15	29.4	1.98, 1.87	29.3	1.98, 1.94
16	26.4	1.91, 0.83	27.2	2.02, 0.90
17	31.4		33.3	
18	42.7	1.63	54.1	1.13 (*d*, 9.2 Hz)
19	43.0	1.38, 0.98	43.5	1.02
20	31.0		39.1	1.05
21	34.5	1.35, 1.13	31.3	1.42, 1.29
22	36.5	1.51, 1.23	40.1	1.40
23	27.9	0.98 (*s*)	27.9	0.97 (*s*)
24	15.0	0.86 (*s*)	15.1	0.85 (*s*)
25	13.7	0.76 (*s*)	13.7	0.74 (*s*)
26	22.1	1.75 (*brs*)	22.1	1.76 (*brs*)
27	18.8	1.57 (*s*)	19.0	1.59 (*s*)
28	27.0	0.84 (*s*)	28.3	0.79 (*s*)
29	33.1	0.88 (*s*)	18.0	0.87 (*d*, 5.7 Hz)
30	24.0	0.88 (*s*)	20.5	0.88 (*d*, 5.7 Hz)

Results and Disccussion

At1g78500 (GenBank number AC007260; T30F21.16), one of the OSC homologous genes of *A. thaliana*, has been shown

(7) (a) Kushiro, T.; Shibuya, M.; Ebizuka, Y. *J. Am. Chem. Soc.* **1999**, *121*, 1208−1216. (b) Kushiro, T.; Shibuya, M., Masuda, K.; Ebizuka, Y. *J. Am. Chem. Soc.* **2000**, *122*, 6816−6824.
(8) Hayashi, H.; Huang, P.; Inoue, K.; Hiraoka, N.; Ikeshiro, Y.; Yazaki, K.; Tanaka, S.; Kushiro, T.; Shibuya, M.; Ebizuka, Y. *Eur. J. Biochem.* **2001**, *268*, 6311−6317.
(9) Shibuya, M.; Adachi, S.; Ebizuka, Y. *Tetrahedron* **2004**, *60*, 6995−7003.
(10) Fazio, G. C.; Xu, R.; Matsuda, S. P. T. *J. Am. Chem. Soc.* **2004**, *126*, 5678−5679.
(11) Xiong. Q.; Wilson, W. K.; Matsuda. S. P. T. *Angew. Chem., Int. Ed.* **2006**, *45*, 1285−1288.
(12) Xiang, T.; Shibuya, M.; Katsube, Y.; Tsutsumi, T.; Otsuka, M.; Zhang, H.; Masuda, K.; Ebizuka, Y. *Org. Lett.* **2006**, *8*, 2835−2838.
(13) Morita, M.; Shibuya, M.; Kushiro, T.; Masuda, K.; Ebizuka, Y. *Eur. J. Biochem.* **2000**, *267*, 3453−3460.
(14) Kushiro, T.; Shibuya, M.; Masuda, K.; Ebizuka, Y. *Tetrahedron Lett.* **2000**, *41*, 7705−7710.



1 2

Figure 1. Structures of **1** and **2**.

to encode a multifunctional triterpene synthase, and some of its products have been identified and reported to be lupeol (**7**), bauerenol (**17**), and α-amyrin (**18**).[15] In addition, this enzyme was found in this study to yield a product(s) with a slightly large R_f value on TLC, which showed two major peaks in HPLC (Figure S1). From a 6 L culture of the yeast transformant, products **1** and **2** (2.7 mg and 1.5 mg, respectively) were isolated upon silica gel column chromatography followed by RP-HPLC.

HREIMS of product **1** gave a molecular ion peak at *m*/*z* 426.3853, indicating its molecular formula to be $C_{30}H_{50}O$ with six degrees of unsaturation. NMR analysis indicated the presence of two double bonds (Table 1). Based on these data, **1** should have a tetracyclic structure. HMQC and HMBC analyses indicated that the two olefins are tri- and tetrasubstituted, respectively. Each of these olefins has only one methyl substitution and is not a part of a dimethylallyl structure, which normally occurs in the side chain terminals of the known tetracyclic triterpenes, suggesting that **1** has a unique tetracyclic skeleton. Further detailed HMQC, HMBC, and DQF-COSY analyses (Figure S3) gave the possible planar structure of **1** (Figure 1).

Product **2** showed very similar NMR spectra to those of **1** (Table 1). Two *geminal* methyl groups in **1** are missing, and in their place the signals of two doublet methyl groups were observed. These spectral differences are reminiscent of the E-ring structures of β-amyrin (**10**) and α-amyrin. Detailed HMBC, HMQC, and DQF-COSY analyses (Figure S3) gave the possible planar structure of **2** (Figure 1).

NOESY analysis of **1** showed correlations between H3 and H5, H5 and H9, and H18 and 28-Me (Figure S4). As eukaryotic OSCs accept only (3*S*)- and not (3*R*)-enantiomer of oxidosqualene as a substrate,[16] the absolute configuration of C3 must be *S*, and thus those of C5 and C9 were determined to be *S*. That of C10 is also *S*, since 25-Me did not show correlations to these protons. In the upper right rings, a correlation between H18 and 28-Me was observed, indicating that the stereochemistry of the ring juncture is (17*R*, 18*R*) or (17*S*, 18*S*). Similarly, the correlations observed by NOESY analysis (Figure S4) indicated that **2** has the configurations 3*S*, 5*S*, 9*S*, and 10*R* in the lower left rings and 17*R*, 18*S*, 19*S*, and 20*R* or 17*S*, 18*R*, 19*R*, and 20*S* in the other decalin system.

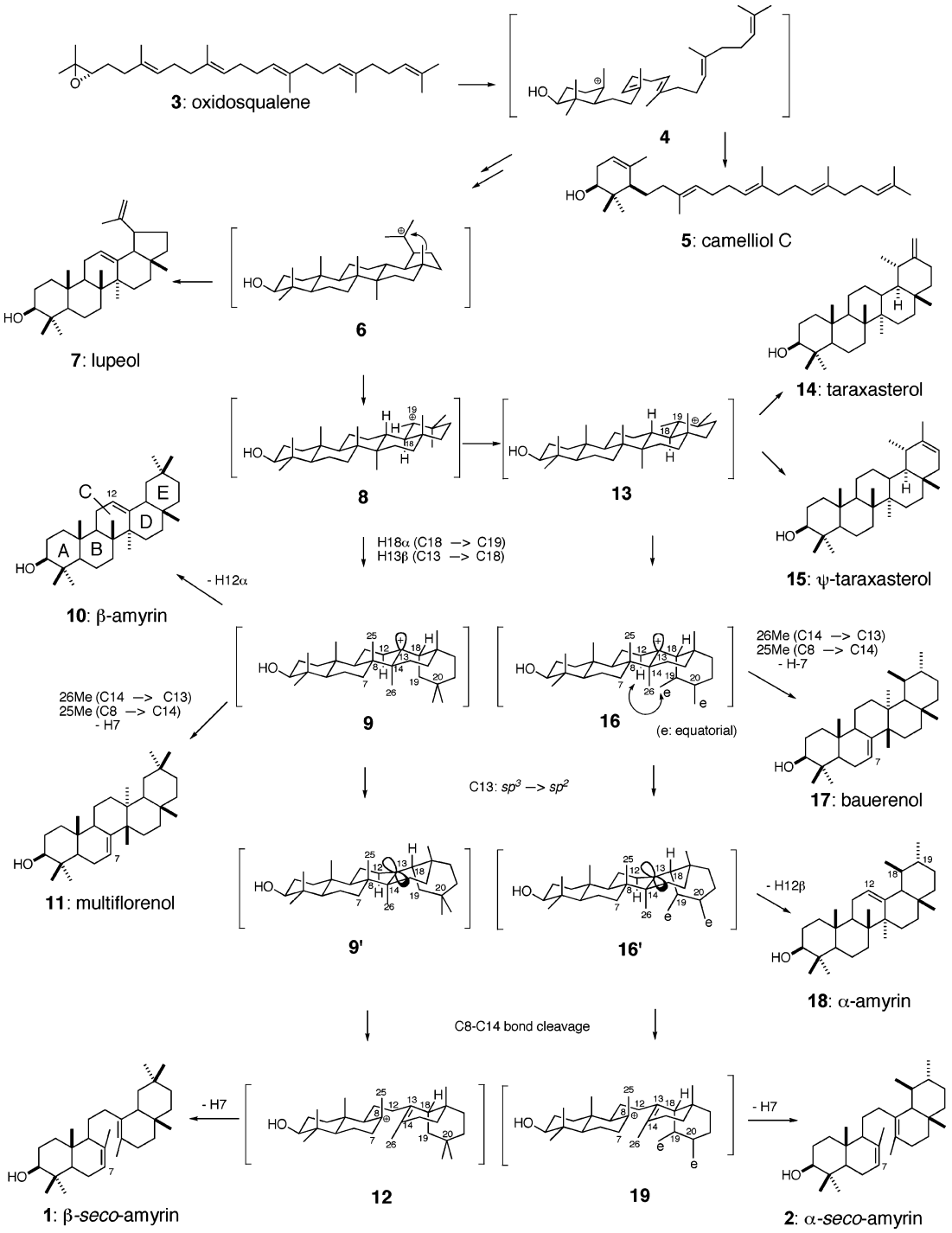
Natural cyclic triterpenes are comprised of mono-, bi-, tri-, tetra-, and pentacyclic structures. These are constructed through successive ring formations initiated by ring opening of an epoxide of oxidosqualene by protonation, following electrophilic addition of the carbocation to the neighboring double bond and the migration of hydrides and methyl groups. In higher plants, triterpenes with 6−6−6−5, 6−6−6−6−5, and 6−6−6−6−6 fused rings occur widely, but to the best of our knowledge, a triterpene with two decalin rings connected by an ethylene bridge, such as in **1** and **2**, has not been previously reported. Although **1** and **2** are the *in vivo* accumulated products of the yeast transformant with At1g78500, their intriguing structures must be constructed by this OSC.

As shown in Scheme 1, diverse 6−6−6−6−6 fused ring systems of natural triterpenes are formed by the so-called backbone rearrangements, starting from all-*chair* pentacyclic cationic intermediate **8** with *antiparallel* 1,2-migration of hydrides and methyl groups, and 1,2-elimination of a proton from the carbon adjacent to the generated cation center. For example, two successive 1,2 hydride shifts (H18α to 19 and H13β to 18) from **8** yield oleanyl cation **9**. In this tertiary cation, the empty orbital of C13 occupies the *anti*-position to H12α, the elimination of which yields β-amyrin (**10**).[17] In the same oleanyl cation **9**, 26-Me also occupies the *anti*-position to the empty orbital and two successive *antiparallel* methyl group shifts (from 14 to 13 and from 8 to 14) with H7 elimination lead to construction of multiflorenol (**11**). If this cationic intermediate **9** had a significant lifetime and the DE-rings were forced to bend slightly upward as a possible consequence of the steric hindrance exerted by the enzyme protein, the empty orbital at C13 would no longer be *anti* to H12α or 26-Me. Instead, it would become nearly *anti* to a *sigma* bond between C8 and C14, and under this stereoelectronic condition the C8−C14 bond would tend to cleave. The intermediate cation **12**, left by a double bond formation between C13 and C14, is quenched by H7 elimination, forming a double bond between C7 and C8 to yield **1**. A similar sequence of reactions from the ursanyl cation could provide **2**.

As mentioned above, we previously reported that this enzyme is multifunctional and its products include lupeol, α-amyrin, and bauerenol.[15] In this study, products that showed the same R_f value on TLC as that of these triterpenes were reanalyzed using GC−MS, instead of LC−MS, to show, in addition to the previously reported products, the presence of taraxasterol (**14**), *pseudo*-taraxasterol (**15**), and multiflorenol, but not β-amyrin (Figure S2). As shown in Scheme 1, the formation of these products can be explained by the quenching of the relevant carbocation intermediates on the way to **1** and **2**. With regard to the elimination of H12, specific elimination of the α-proton from the oleanyl cation and the β-proton from the ursanyl cation were demonstrated by feeding [5-^{13}C, $^{2}H_2$] mevalonate to the cell suspension cultures of *Rabdosia japonica*.[17] The lack of β-amyrin formation by this OSC may point to the preferred sp^2-like structure at C13 of cation **9′** as proposed, since the H12α is no longer *anti* to an empty orbital at C13. In the proposed sp^2-like ursanyl cation **16′**, a branching point between α-amyrin and **2** formation, the *sigma* bond between C8 and C14 becomes nearly *anti* to an empty orbital at C13 and the cleavage of this bond leads to the formation of **2**. In contrast, both H12α and H12β of **16′** are now *gauche* to an empty orbital at C13, and in addition, the β-methyl group at C19 of the E-ring, which in **16** is interfering with access of a base from the α-side of C12 and renders anomalous *syn*-elimination from the β-side for α-amyrin

(15) Ebizuka, Y.; Katsube, Y.; Tsutsumi, T.; Kushiro, T.; Shibuya, M. *Pure Appl. Chem.* **2003**, *75*, 361−366.
(16) Barton, D. H. R.; Jarman T. R.; Watson K. C.; Widdowson D. A.; Boar R. B.; Damps K. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1134−1138.
(17) Seo, S.; Yoshimura, Y.; Uomori, A.; Takeda, K.; Seto, H.; Ebizuka, Y.; Sankawa, U. *J. Am. Chem. Soc.* **1988**, *110*, 1740−1745.

Scheme 1. Proposed Reactions of Oxidosqualene Cyclases

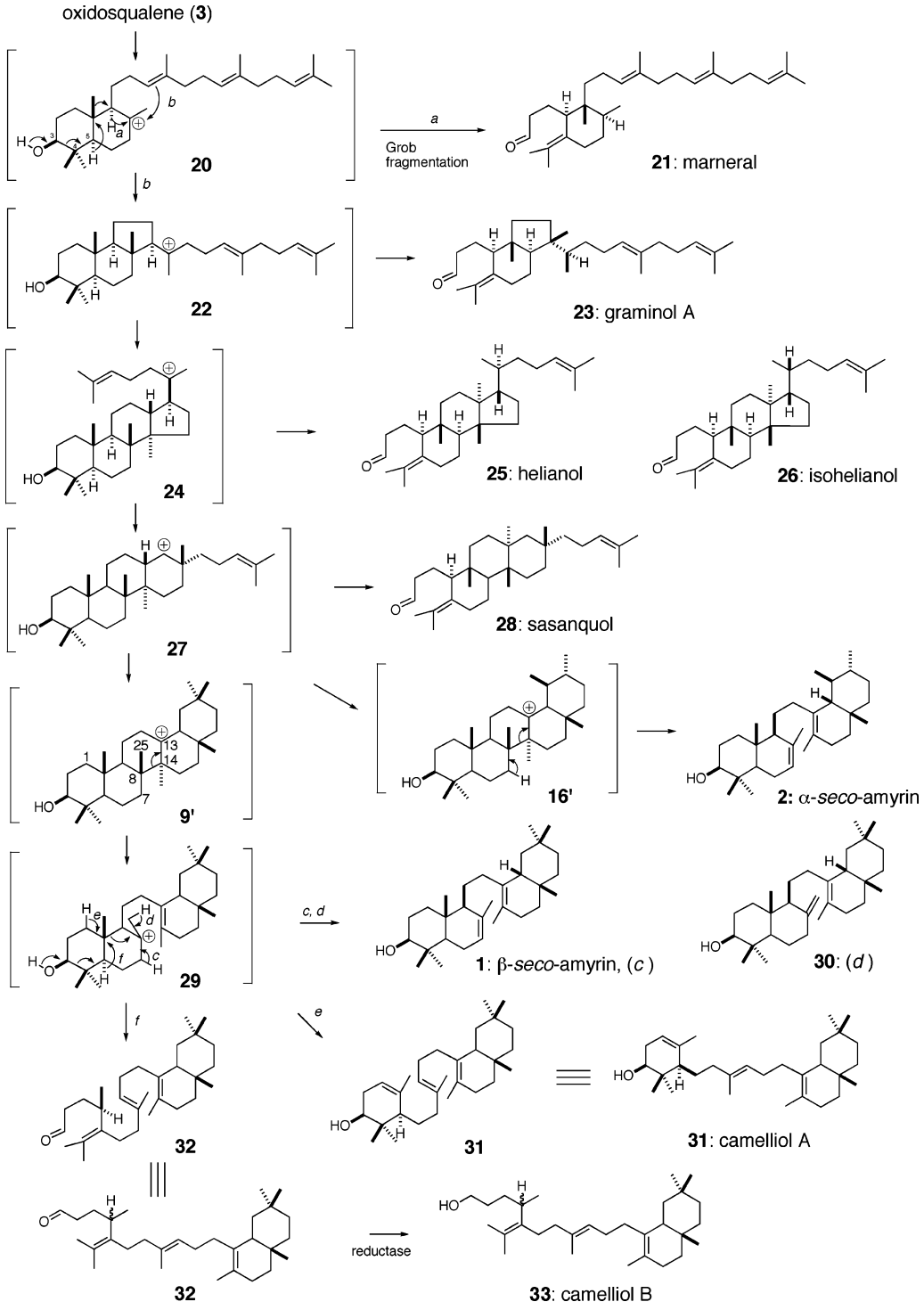


formation, no longer interferes with access of a base to eliminate a β-proton from C12. It is intriguing to know which of the C12 protons of the intermediate **16′** is eliminated to produce α-amyrin by this OSC. With regard to the C8−C14 bond fragmentation, the cleaved bond could migrate, instead of undergoing double bond formation, to C12, forming a 6−6−5−6−6 *spiro* ring system and leaving a cation center on C14. Although this ring system has never been reported as a natural product, it is tempting to speculate that there might be a triterpene with this ring system among the so far unidentified triterpene byproducts of this OSC.

If **1** and **2** are produced by the mechanism discussed above, the stereochemistry of the DE-rings of these compounds must be identical to that of β-amyrin and α-amyrin and, thus, be 17*R* and 18*R* in **1** and 17*R*, 18*S*, 19*S*, and 20*R* in **2**. These compounds are new natural products and named β-*seco*-amyirn and α-*seco*-amyrin, respectively. A triterpene (**30**) with a structure similar to that of β-*seco*-amyrin has been reported from *Stevia viscida* and *S. eupatoria*,[18] which has an *exo*-methylene at C8 in place

(18) Román, L. U.; Guerra-Ramírez, D.; Morán, G.; Martínez, I.; Hernández, J. D.; Cerda-García-Rojas, C. M.; Torres-Valencia, J. M.; Joseph-Nathan, P. *Org. Let.* **2004**, *6*, 173−176.

Scheme 2. Proposed Reactions for Natural *seco*-Triterpene Formation by Oxidosqualene Cyclases



of a double bond between C7 and C8 in **1**. This compound must be derived from the same intermediate cation **29** by elimination of a proton from the 26-methyl group (Scheme 2, *d*).

Recently, Xiong et al. reported the identification of another OSC from *A. thaliana*, At5g42600, as a marneral synthase[11] yielding the A-ring-*seco*-monocyclic triterpene **21**. Grob fragmentation of the A-ring in bicyclic carbocation at C5 generated by hydride and methyl group shifts cleaves the carbon−carbon bond between C3 and C4 and leaves an aldehyde functionality at C3 (Scheme 2, *a*). In the case of the At1g78500-catalyzed reaction, carbocation returns to C13 of the C-ring in a series of *antiparallel* hydride and/or methyl group shifts from pentacyclic carbocation intermediates, and cleavage of the carbon−carbon bond between C8 and C14 to form a C13−C14 double bond yields C-ring-*seco*-amyrins. OSCs have been believed to catalyze extensive carbon−carbon bond formations and rearrangements including hydride and methyl group shifts but not recognized to catalyze these fragmentation reactions. The discovery of marneral synthase and an OSC yielding *seco*-amyrins in this study revealed that OSCs catalyze not only the formation of fused multiring systems in an earlier part of the reaction course but also fragmentations of self-built-up ring

systems in a later part to elaborate *seco*-triterpenes. Extension of these discussions would provide a rational explanation for the formation of some naturally occurring *seco*-triterepnes by OSCs. As shown in Scheme 2, graminol A (**23**),[19] helianol (**25**),[20] isohelianol (**26**)[20] and sasanquol (**28**)[21] have been suggested to be synthesized by Grob fragmentation from the corresponding 6−6−5, 6−6−6−5, and 6−6−6−6 fused ring intermediate cations (**22**, **24**, **27**) formed after the return of the cation center to the A-ring.[11] Further interesting *seco*-triterpene structures include those reported from *Camellia* sp., *i.e.*, camelliols A (**31**) and B (**33**).[22]

Camelliol A has a structure in which a cyclohexene ring and a decalin ring system are connected with a methylhexene bridge, and camelliol B has a decalin ring system with a branched C_{16} side chain. Thus, neither of these structures looks like an OSC product, and it seems difficult to elucidate their biosynthetic derivations. The co-occurrence of camelliol C (**5**) in the same source[22] makes the situation further confusing, since the OSC-catalyzed formation of the monocyclic triterpene camelliol C is easily understood as it can be derived from the monocyclic cation intermediate **4** by deprotonation from C1 leaving the other end of the substrate, oxidosqualene, intact. In light of the discussions above, the decalin ring system found in both of these structures reminds us of that of **1**, and the terminal branched aliphatic structure of camelliol B of those produced by Grob fragmentation catalyzed by OSCs. Taking all the information together, we now propose that camelliol A is a product of OSC by two successive cleavages of the C- and B-rings from the oleanyl cation and deprotonation from C1 (Scheme 2, *e*); similarly the aldehyde **32** corresponding to camelliol B is a product of Grob fragmentation from the same intermediate cation **29** in the A-ring (Scheme 2, *f*). Camelliol B itself may be derived from aldehyde **32** by an endogenous reductase in the original plant. These *seco*-triterpenes are found in the seed oil of plants belonging to Theaceae and Gramineae. cDNA cloning from plants in these families is now underway to confirm the presence of OSCs yielding these *seco*-triterpenes.

A unique OSC yielding C-ring *seco*-triterpenes was found in *A. thaliana* OSC homologues in this study. Together with the Grob fragmentation reported for the production of marneral (**21**) by an OSC, the formation of *seco*-amyrins by the present OSC revealed that OSCs have the ability to cleave preformed ring systems in addition to forming fused multiring systems. These findings led to a careful survey of natural *seco*-triterpene structures and enabled us to propose that some natural *seco*-triterpenes, for which the biosynthetic mechanism has not been elucidated, are direct products of OSCs. The inclusion of these *seco*-triterpene skeletons further increased the diversity of structures produced by OSCs than we previously recognized.

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Supporting Information Available: HMBC, DQF-COSY, and NOESY correlations, spectra of ^{1}H and ^{13}C NMR, and profiles of LC and GC. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) Akihisa, T.; Koike, K.; Kimura, Y.; Sashida, N.; Matsumoto, T.; Ukiya, M.; Nikaido, T. *Lipids* **1999**, *34*, 1151−1157.
(20) Akihisa, T.; Kimura, Y.; Koike, K.; Shibata, T.; Yoshida, Z.; Nikaido, T.; Tamura, T. *J. Nat. Prod.* **1998**, *61*, 409−412.
(21) Akihisa, T.; Yasukawa, K.; Kimura, Y.; Yamanouchi, S.; Tamura, T. *Phytochemistry* **1998**, *48*, 301−305.
(22) Akihisa, T.; Arai, K.; Kimura, Y.; Koike, K.; Kokke, W. C. M. C.; Shibata, T.; Nikaido, T. *J. Nat. Prod.* **1999**, *62*, 265−268.