Available online at www.sciencedirect.com

SCIENCE DIRECT®

ELSEVIER

Tetrahedron Letters

Tetrahedron Letters 45 (2004) 8299–8301

Enzymatic formation of an unnatural novel tetracyclic sesterterpene by β-amyrin synthase

Hisashi Noma,[a] Hideya Tanaka,[a] Hiroshi Noguchi,[a] Masaaki Shibuya,[b] Yutaka Ebizuka[b] and Ikuro Abe[a,*]

[a]*School of Pharmaceutical Sciences and the 21st Century COE Program, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan*

[b]*Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*

Received 5 August 2004; revised 9 September 2004; accepted 10 September 2004
Available online 28 September 2004

Abstract—A convergent synthesis provided a C_{25} and a C_{35} oxidopolyprene in which a farnesyl C_{15} unit is connected in a head-to-head fashion to a geranyl C_{10} or a geranylgeranyl C_{20} unit. When incubated with recombinant β-amyrin synthase from *Pisum sativum* the C_{25} oxidopolyprene was enzymatically converted to an unnatural novel tetracyclic sesterterpene, while the C_{35} analogue did not afford any cyclization product.
© 2004 Elsevier Ltd. All rights reserved.

β-Amyrin synthase (βAS) (E.C. 5.4.99.-) catalyzes the remarkable conversion of (3*S*)-2,3-oxidosqualene (**1**) to β-amyrin (**2**), forming a 6/6/6/6/6-fused ring system with a total of eight chiral centers in a single reaction.[1] The proton-initiated cyclization first produces the tetracyclic dammarenyl C-20 cation, and the subsequent skeletal rearrangements and 1,2-hydride shifts lead to formation of the pentacyclic framework with the Δ^{12} double bond (Scheme 1A). Interestingly, pentacyclic triterpene synthases accept a variety of non-physiological substrate and efficiently performs sequential ring-forming reactions to produce a series of unnatural cyclic triterpenoids. We have previously demonstrated that recombinant βAS from *Pisum sativum* accepted 22,23-dihydro-2,3-oxidosqualene (**3**) to yield a 4:1 mixture of euph-7-en-3β-ol (**4**) and bacchar-12-en-3β-ol (**5**) (Scheme 1B).[2] Further, the enzyme efficiently converted 24,30-bisnor-2,3-oxidosqualene into 29,30-bisnor-β-amyrin and its regioisomers.[3] On the other hand, it has been recently reported that lupeol synthase from *Arabidopsis thaliana* cyclized non-physiological 3-(ω-oxidogeranylgeranyl)-indole into petromindole, an indole diterpene with a 6/6/6/6/6/5-fused ring system.[4] Moreover, squalene:hopene cyclase from a thermoacidophilic bacteria *Alicyclobacillus acidocaldarius* accepted a C_{35} (geranylgeranyl-farnesyl) polyprene hydrocarbon as a substrate to yield an unnatural C_{35} hexacyclic polyprenoid with a 6/6/6/6/6/5-fused ring system.[5] Here in this paper, to further explorer the biosynthetic potential of the βAS enzyme, we tested enzymatic conversion of newly synthesized C_{25} and C_{35} oxidopolyprene in which a farnesyl C_{15} unit is connected in a head-to-head fashion to a geranyl C_{10} or a geranylgeranyl C_{20} unit.

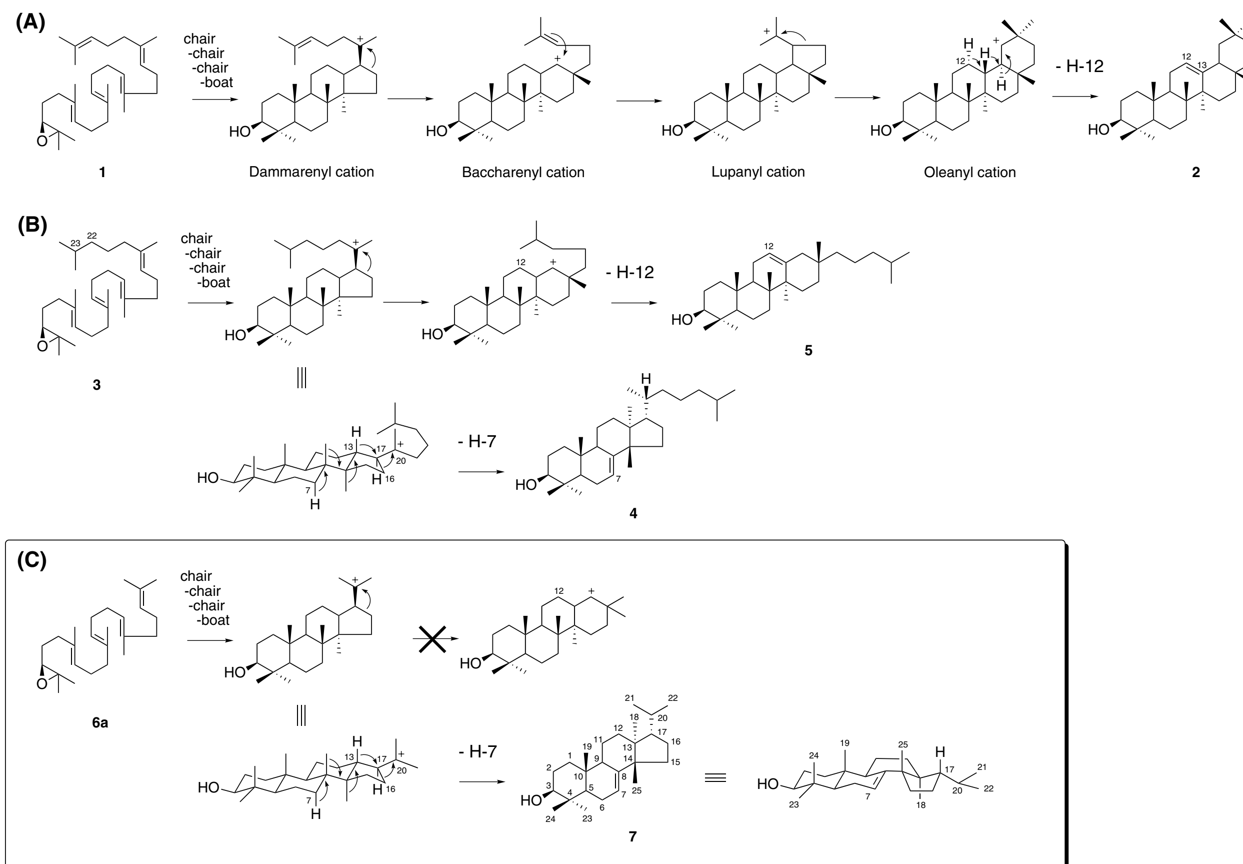
The convergent synthesis of the C_{25} (**6a**)[6] and the C_{35} (**6b**)[7] oxidopropyrene (both in racemic form) involved the coupling of oxidofarnesyl phenylsulfone with geranyl or geranylgeranyl bromide, which was followed by dephenylsulfonation by $LiHBEt_3$ in the presence of catalytic amount of $PdCl_2$ [1,3-bis(diphenylphosphino)propane] as described before (Scheme 2).[5]

When enzymatic conversion of the C_{35} oxidopolyprene (**6b**) was attempted with the recombinant *P. sativum* βAS,[8] no cyclization product could be detected by TLC or GC. Thus, the C_{35} oxidopolyprene was not accepted as a substrate, suggesting that the catalytic site of the βAS does not have enough space to accept the larger substrate analogue with an additional C_5 isoprene unit. In contrast, enzyme reaction with the C_{25} oxidopolyprene (**6a**) resulted in isolation of a single cyclization product (0.3 mg, 0.3% yield).[8] Spectroscopic data (^{1}H and ^{13}C NMR, HMQC, HMBC, and MS)[9] of the

Keywords: β-Amyrin synthase; Oxidosqualene cyclase; Triterpene synthase; Unnatural sesterterpene.

* Corresponding author. Tel./fax: +81 54 264 5662; e-mail: abei@ys7.u-shizuoka-ken.ac.jp

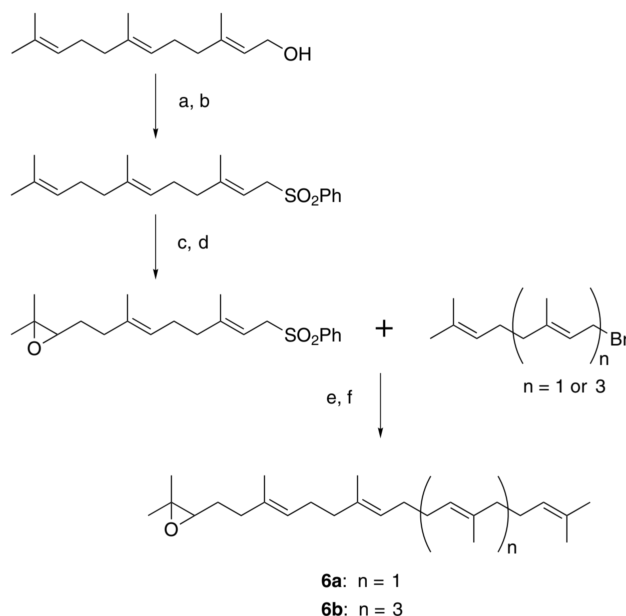
0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.09.075



Scheme 1. Proposed mechanism for the conversion of (A) 2,3-oxidosqualene (**1**) to β-amyrin (**2**); (B) 22,23-dihydro-2,3-oxidosqualene (**3**) to a 4:1 mixture of euph-7-en-3β-ol (**4**) and bacchar-12-en-3β-ol (**5**); (C) C_{25} oxidopropyrene (**6a**) to compound **7** by recombinant *P. sativum* β-amyrin synthase.

product were characteristic of those of tetracyclic hydrocarbon alcohols, and showed good agreement with euph-7-en-3β-ol (**4**)[2] except the signals due to the alkyl side chain, suggesting the structure of a novel tetracyclic sesterterpene alcohol (**7**) (Scheme 1C). The 6/6/6/5 ring system was uniquely consistent with both biogenetic reasoning and the heteronuclear correlation NMR spectroscopy (HMQC and HMBC). Finally, the stereochemistry of C-17 was confirmed by NOEs observed between Me-19/Me-24 and Me-19/Me-25, but absent between Me-18/Me-25 and Me-25/Me-21,22.

The enzymatic conversion of the C_{25} oxidopolyprene (**6a**) (0.3% yield) by the recombinant βAS was much less efficient than that of 22,23-dihydro-2,3-oxidosqualene (3%)[2] and 24,30-bisnor-2,3-oxidosqualene (30%).[3] The absence of the terminal C_5 isoprene unit of 2,3-oxidosqaulene thus resulted in the poor yield of the cyclization reaction. As in the case of the enzymatic formation of euph-7-en-3β-ol (**4**) from 22,23-dihydro-2,3-oxidosqualene (**3**), a substrate analogue lacking the terminal double bond of 2,3-oxidosqualene (Scheme 1B), βAS initiated cyclization of **6a** from a *chair–chair–chair–boat* conformation to generate the Markovnikov dammarene-type tertiary cation with the 17β-isopropyl group. Then, a backbone rearrangement (H-17α → 20α, H-13β → 17β, CH_3-14α → 13α, CH_3-8β → 14β) with elimination of H-7α proton yielded the unnatural tetracyclic



Scheme 2. Reagents and conditions: (a) PBr_3, hexane, −5 °C; (b) $PhSO_2$, DMF, 83%; (c) NBS, 20% THF/H_2O, 28%; (d) K_2CO_3, MeOH, 88%; (e) *n*-BuLi (1 equiv), THF, −78 °C, 72%; (f) $[PdCl_2(dppp)]$ (10 mol%), $LiBEt_3H$ (2 equiv), 0 °C, 58%.

product **7** with a Markovnikov five-membered D-ring (Scheme 1C). It was remarkable that the stereochemistry of the non-physiological product, whose structure is apparently different from β-amyrin, was strictly controlled by the enzyme, even in the absence of the terminal C_5 isoprene unit. Interestingly, no evidence was obtained for the formation of baccharene-type product with the anti-Markovnikov six-membered D-ring in the reaction mixture. Further analyses of the catalytic potential of functionally divergent triterpene synthases promise to reveal intimate structural details of the enzyme catalyzed processes and suggest strategies for manipulating substrate and product specificities of the polyene cyclization reactions.

Acknowledgements

This work was in part supported by the 21st Century COE Program and Grant-in-Aid for Scientific Research (Nos. 16510164, 1531053, and 15101007) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and by Grant-in-Aid from The Mochida Memorial Foundation for Medical and Pharmaceutical Research, and the Tokyo Biochemical Research Foundation, Japan.

Supplementary data

Complete set of spectroscopic data (^{1}H and ^{13}C NMR, HMQC, HMBC, NOE, and MS) of compound **7** (7 pages). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2004.09.075.

References and notes

1. For reviews, see: (a) Abe, I.; Rohmer, M.; Prestwich, G. D. *Chem. Rev.* **1993**, *93*, 2189–2206; (b) Wendt, K. U.; Schulz, G. E.; Corey, E. J.; Liu, D. R. *Angew. Chem., Int. Ed.* **2000**, *39*, 2812–2833; (c) Xu, R.; Fazio, G. C.; Matsuda, S. P. T. *Phytochemistry* **2004**, *65*, 261–291.
2. Abe, I.; Sakano, Y.; Tanaka, H.; Lou, W.; Noguchi, H.; Shibuya, M.; Ebizuka, Y. *J. Am. Chem. Soc.* **2004**, *126*, 3462–3463.
3. Abe, I.; Sakano, Y.; Sodeyama, M.; Tanaka, H.; Noguchi, H.; Shibuya, M.; Ebizuka, Y. *J. Am. Chem. Soc.* **2004**, *126*, 6880–6881.
4. Xiong, Q.; Zhu, X.; Wilson, W. K.; Ganesan, A.; Matsuda, S. P. T. *J. Am. Chem. Soc.* **2003**, *125*, 9002–9003.
5. Abe, I.; Tanaka, H.; Noguchi, H. *J. Am. Chem. Soc.* **2002**, *124*, 14514–14515.
6. C_{25} oxidopolyprene **6a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.13 (m, 4H), 2.70 (t, 1H, J = 6.2 Hz), 2.09–1.98 (m, 16H), 1.68 (s, 3H), 1.60 (s, 9H), 1.55 (s, 3H), 1.30 (s, 3H), 1.26 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 135.1, 134.9, 134.0, 131.2, 124.9, 124.4 (×2), 124.3, 64.2, 58.3, 39.7, 39.7, 36.3, 28.2 (×2), 27.5, 26.7, 26.7, 25.7, 24.9 (×2), 18.7, 17.7, 16.0, 16.0. HRMS (EI): found for [$C_{25}H_{42}O$] 358.3240; calcd 358.3236.
7. C_{35} oxidopolyprene **6b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.13 (m, 6H), 2.70 (t, 1H, J = 5.6 Hz), 2.20–1.96 (m, 24H), 1.68 (s, 3H), 1.60 (s, 15H), 1.55 (s, 3H), 1.30 (s, 3H), 1.26 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 135.1, 134.9 (×2), 134.9, 134.0, 131.2, 124.9, 124.8, 124.4 (×2), 124.3 (×2), 64.2, 58.3, 39.8, 39.7 (×3), 36.3, 28.3 (×2), 27.5, 26.8, 26.7 (×3), 25.7, 24.9, 18.7, 17.7, 16.0 (×3), 16.0 (×2). HRMS (EI): found for [$C_{35}H_{58}O$] 494.4472; calcd 494.4488.
8. *P. sativum* βAS was expressed in the yeast mutant strain GIL77 (10 L of culture) as described before.[2,3] The reaction mixture containing 100 mg of C_{25} oxidopolyprene **6a** (or C_{35} oxidopolyprene **6b**), 0.45 M sucrose, 1 mM EDTA, 1 mM DTT, and 0.1% Triton X-100 in 200 mL of 0.1 M KPB, pH 7.4, was incubated at 30 °C for 18 h. The incubations were stopped by adding equal volume of 20% KOH in 50% aq EtOH, saponified at 30 °C for 24 h, and extracted with 400 mL of hexane (×3). The combined extracts were evaporated to dryness, separated on SiO_2 column (15% EtOAc/hexane), and finally purified by HPLC (YMC-Pack PRO C4; 5% THF/CH_3CN; 0.3 mL/min) to give 0.3 mg of compound **7**, along with 3.5 mg of β-amyrin derived from 2,3-oxidosqualene accumulated in the mutant yeast cells.
9. Compound **7**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.26 (dt, 1H, J = 4.0, 2.8 Hz, H-7), 3.24 (dd, 1H, J = 11.0, 4.2 Hz, H-3), 0.97 (s, 6H, Me-23, Me-25), 0.88 (d, 6H, J = 6.4 Hz, Me-21, Me-22), 0.86 (s, 3H, Me-24), 0.81 (s, 3H, Me-18), 0.75 (s, 3H, Me-19); ^{13}C NMR (100 MHz, $CDCl_3$): δ 146.9 (C-8), 117.8 (C-7), 79.2 (C-3), 54.9 (C-17), 51.2 (C-14), 50.6 (C-5), 48.9 (C-9), 43.5 (C-13), 38.9 (C-4), 37.2 (C-1), 35.0 (C-10), 34.0 (C-15), 33.5 (C-12), 29.7 (C-20), 28.3 (C-16), 27.7 (C-23), 27.6 (C-25), 24.0 (C-6), 22.2 (C-21), 22.2 (C-22), 21.9 (C-18), 18.0 (C-11), 14.7 (C-24), 13.1 (C-19). The NMR assignments were performed according to data from HMQC, HMBC experiments, and by comparison with the data of euph-7-en-3β-ol. LRMS (EI): m/z 358 $[M]^+$, 343, 325, 203, 175, 119, 95, 69, 43. HRMS (EI): found for [$C_{25}H_{42}O$] 358.3241; calcd 358.3236.