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Synthesis and Enzymatic Cyclization of (3*S*)11-Fluoro-2,3-oxidosqualene

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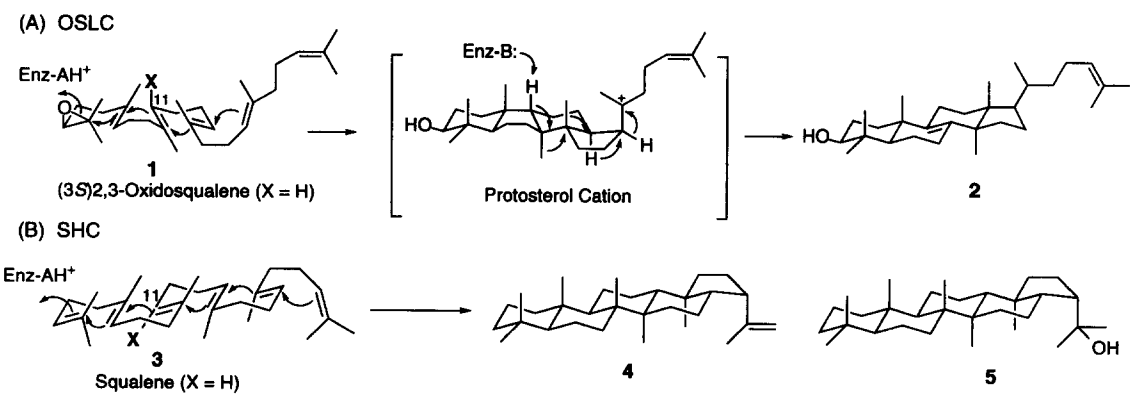
Abstract: A convergent asymmetric synthesis provided (3*S*)11-fluoro-2,3-oxidosqualene (11-FOS, **14**), which was cyclized by bacterial squalene:hopane cyclase to a bridged ether. 11-FOS was neither a substrate nor an inhibitor for vertebrate oxidosqualene:lanosterol cyclase.

Oxidosqualene:lanosterol cyclase (OSLC) (E.C. 5.4.99.7) and bacterial squalene:hopene cyclase (SHC) (E.C. 5.4.99.7) catalyze remarkable carbon-carbon bond-forming reactions in the biosynthesis of sterols and triterpenes.[1] The enzymes bind the substrate folded in chair-boat-chair (OSLC) or in all chair (SHC) conformation and then mediate sequential ring-forming reactions and rearrangements through a progression of rigidly-held carbocationic intermediates (Scheme 1). These membrane-associated 70-85 kDa proteins show 17-27% sequence identity between the bacterial and eukaryotic proteins.[2,3] Both SHC and OSLC contain six repeats of a highly-conserved α-helix turn motif rich in aromatic amino acids (the QW motif).[4] Recently, the three-dimensional structure of SHC from a thermoacidophilic bacteria *Alicyclobacillus acidocaldarius* was reported.[3d] In this paper, we report the synthesis and enzymatic cyclization of (3*S*)11-fluoro-2,3-oxidosqualene (11-FOS, **14**) in which 11-H has been replaced by a fluorine atom. The dramatic effect of fluorine was apparent from the analysis of the major cyclization product obtained with purified recombinant *A. acidocaldarius* SHC, and from the absence of cyclization of 11-FOS by rat liver OSLC.

The convergent synthesis of 11-FOS involved the Sharpless asymmetric dihydroxylation[5] of fluoroester **7** and the coupling of α-fluoro allylic bromide **10** with farnesyl phenylsulfone **12** as the key steps (Scheme 2). The



Scheme 1. Proposed mechanism for the cyclization of (3*S*)2,3-oxidosqualene (**1**) to lanosterol (**2**) by OSLC (**A**) and squalene (**3**) to hop-22-ene (**4**) and hopan-22-ol (**5**) by SHC (**B**).

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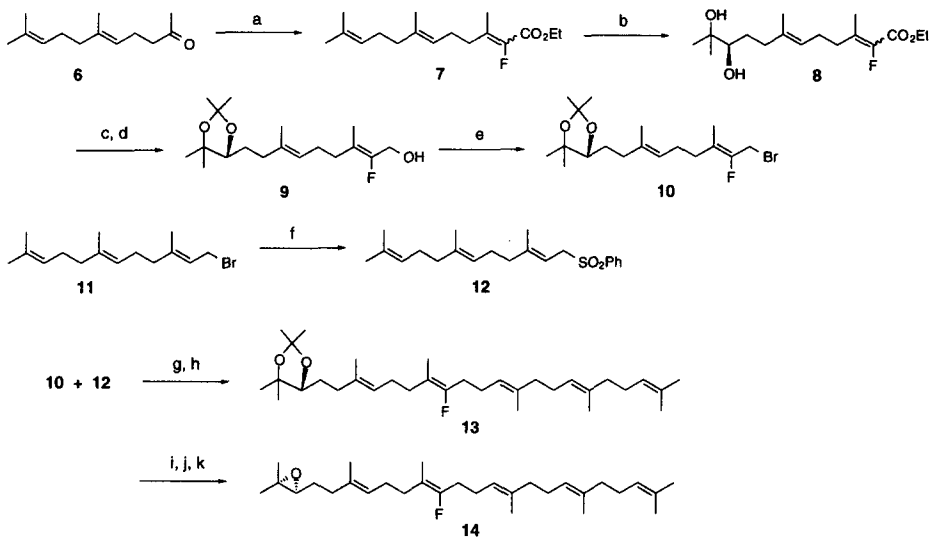

Scheme 2. (**a**) $(EtO)_2P(O)CHFCO_2Et$, NaH, THF, >95%; (**b**) AD-mix-β, 31%, based on recovered **7**; (**c**) 2,2-dimethoxypropane, PPTS, CH_2Cl_2, 85%; (**d**) $LiAlH_4$, THF, 86%; (**e**) PBr_3, hexane, 68%; (**f**) $PhSO_2Na$, DMF, 79%; (**g**) *n*-BuLi, THF, -78 °C, 54%; (**h**) $PdCl_2$[dppp], $LiHBEt_3$, 63%; (**i**) TsOH, 66%; (**j**) DMAP, MsCl, TEA, CH_2Cl_2, 86%; (**k**) K_2CO_3, MeOH, 93%.

fluoroester **7** was obtained from geranyl acetone **6** by the Horner-Wadsworth-Emmons reaction, and the (2*Z*) and (2*E*) isomers were most readily separated for fluoroalcohol **9**; the geometry of the fluoroolefin was established by NOESY and TOCSY experiments. After the coupling reaction and dephenylsulfonation, **13** was deprotected to give a chiral diol, and the epoxide was closed to give the (3*S*)11-FOS, **14**.[6]

When cyclization of 11-FOS was attempted with purified rat liver OSLC, no cyclization product could be detected by TLC or GLC.[7] The OSLC enzyme is particularly sensitive to structural changes on the pro-β-face and thus fails to bind (3*S*)11-FOS.[1] Indeed, (*RS*)11-FOS (as a mixture of regioisomers) did not inhibit OSLC (IC_{50} > 400 μM).[8] In contrast, recombinant *A. acidocaldarius* SHC converted (3*S*)11-FOS into a carbocyclic compound with a bridged ether **15** in 27% isolated yield.[9,10] The ^1H NMR spectrum of this product showed the presence of three methyl singlets (δ 1.33, 1.05, 1.02), five vinylic methyl groups, three vinylic protons, and a proton geminal to the ether bridge (δ 3.72, d, *J* = 5.5 Hz). Other spectroscopic data (HMQC, HMBC, and MS) were also uniquely consistent with structure **15**. Similar mono-carbocyclic compounds with the bridged ether structure have been obtained by acid-induced non-enzymatic cyclization reaction of polyenes.[11] No evidence was found for bi-, tri-, tetra-, or pentacarbocyclic products in the reaction mixture.[12]

For SHC, 11-FOS was accepted in the catalytic site, but the presence of the fluorine atom interrupted the cyclization reaction at the monocyclic cationic intermediate stage; intramolecular trapping by the 3β-OH led to a

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Figure 1. Structure of the cyclization product (left) and the bond connectivities established by HMBC and HMQC spectra (right).

bridged bicyclic ether.[13] Partial cyclization may be attributed to the strong electron-withdrawing effect of the 11-F atom α to an incipient cationic site. Alternatively, the slightly larger fluorine could perturb the optimal folding conformation of the substrate. It is noteworthy that the cyclization of (3*S*)11-FOS by SHC was directional; that is, cyclization was initiated by oxirane ring opening and not by a proton attack on the terminal double bond. Similar results have been observed for the cyclization of oxidosqualene by bacterial squalene cyclase.[12,14]

Recently, Johnson developed a non-enzymatic polyene cyclization reaction using fluorine atom as a cation-stabilizing auxiliary that served to both enhance the cyclization reaction and control the regiochemistry of the product.[15] The synthesis and cyclization of additional fluorinated oxidosqualene analogs will be reported in due course.

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6. (**a**) B. Robustell, M.Sc. Thesis, State University of New York at Stony Brook, Stony Brook, 1996; (**b**) (3*S*)11-FOS: ^{1}H-NMR (300 MHz, $CDCl_3$): δ 5.20-5.06 (m, 4H), 2.71 (t, J = 6.3 Hz, 1H), 2.18-1.92 (m, 20H), 1.68 (s, 3H), 1.60-1.57 (15H), 1.31 (s, 3H), 1.26 (s, 3H). ^{13}C-NMR (63 MHz, $CDCl_3$): δ 154.4 (d, J = 241.9 Hz), 136.1, 135.0, 134.3, 131.3, 124.6, 124.3, 124.1, 122.9, 111.1 (d, J =17.2 Hz), 64.2, 58.3, 39.7 (× 2), 36.2, 29.6 (d, J = 7.5 Hz), 28.8 (d, J = 29.1 Hz), 27.4, 26.7, 26.6, 26.2, 25.7, 25.2, 24.9, 18.7, 17.7, 15.9 (× 3), 15.6 (d, J = 6.5 Hz). ^{19}F-NMR (235 MHz, $CDCl_3$): δ -113.8 (t, J = 21.5 Hz). HRMS (FAB) (Mass Spectroscopy Laboratory, University of Illinois at Urbana-Champaign): found for $C_{30}H_{50}FO$ (MH^+) 445.3836; calcd. 445.3840.

7. Rat OSLC was purified from 500 g of liver according to the published method.[2a] (3*S*)11-FOS (5.0 mg) was incubated with the enzyme in 100 mL of 100 mM Tris-HCl, pH 7.4, 0.1% Triton X-100 at 37 °C for 16 h. After extraction with EtOAc (300 mL × 2), no product was detected and (3*S*)11-FOS (4.6 mg) was recovered unchanged. This was also confirmed by GC analysis.
8. (**a**) 11-Fluorosqualene (11-FS) was first prepared as a pseudosubstrate for squalene epoxidase, but failed to be epoxidized enzymatically: S. Sen, Ph.D. Dissertation, State University of New York at Stony Brook, Stony Brook, 1989; (**b**) An inseparable mixture of racemic 11-F- and 14-F- OS regioisomers was first chemically synthesized from 11-FS: Xiao, X.-y. Ph.D. Dissertation, State University of New York at Stony Brook, Stony Brook, 1991.
9. The recombinant *A. acidocaldarius* SHC was expressed in *E. coli* and purified as described.[3b,e] The enzyme converted squalene into a 17:1 mixture of **4** and **5**, and showed an apparent K_M = 1.6 μM and k_{cat} = 2.4 min^{-1}. The reaction mixture contained (3*S*)11-FOS (4.0 mg) and SHC (60 mg) in 100 mL of 50 mM Na-citrate, pH 6.0, 0.1% Triton X-100, and was incubated at 60 °C for 16 h. The incubations were stopped by freezing and lyophilization, followed by extraction with EtOAc (300 mL × 2). The combined extracts were evaporated to dryness, and purified by SiO_2 TLC (10% EtOAc/Hexane, R_f = 0.4) to give 1.1 mg of compound **15**. Control experiments were carried out at 4 °C or without enzyme preparation.
10. Compound **15**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.20-5.06 (m, 3H), 3.72, (d, *J* = 5.5 Hz, 1H), 1.68 (s, 3H), 1.61 (s, 3H), 1.60 (s, 6H), 1.56 (s, 3H), 1.33 (s, 3H), 1.05 (s, 3H), 1.02 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 154.2 (d, *J* = 240.3 Hz), 136.2, 135.0, 131.3, 124.4, 124.1, 122.9, 111.4 (d, *J* = 17.0 Hz), 86.7, 86.1, 55.3, 45.2, 39.7 (× 2), 39.0, 29.7 (d, *J* = 7.5 Hz, 28.8 (d, *J* = 29.3 Hz), 26.8, 26.6, 26.0, 25.8, 25.7, 25.5, 25.3, 23.3, 18.8, 17.7, 16.0, 15.4 (d, *J* = 5.1 Hz). LRMS (EI, 80 eV): *m/z* 444 (M^+, 5), 153 (92), 135 (71), 109 (43), 95 (67), 81 (76), 69 (100). HRMS (EI, 80 eV): found for $C_{30}H_{49}FO$ 444.3754; calcd. 444.3767. Complete spectral data may be requested from the authors.
11. For example, see: (**a**) van Tamelen, E. E.; Coates, R. M. *Bioorg. Chem.* **1982**, *11*, 171; (**b**) Fish, P. V.; Johnson, W. S. *J. Org. Chem.* **1994**, *59*, 2324.
12. Under the same conditions, the recombinant *A. acidocaldarius* SHC converted (3*S*)2,3-oxidosqualene to 3β-hydroxyhop-22(29)-ene and hopan-3β,22-diol (I. Abe, unpublished data).
13. Cyclization reaction of (3*R*)2,3-oxidosqualene by squalene cyclase from protozoan *Tetrahymena pyriformis* was also interrupted at the monocyclic stage and afforded a monocyclic triterpene with 2,3,4-trimethyl-cyclohexanone structure.[14d]
14. Cell-free homogenates of hopanoid-producing microorganisms initiate cyclization reactions of oxidosqualene into pentacyclic triterpenes by oxirane ring opening. See: (**a**) Rohmer, M.; Anding, C.; Ourisson, G. *Eur. J. Biochem.* **1980**, *112*, 541; (**b**) Rohmer, M.; Bouvier, P.; Ourisson, G. *Eur. J. Biochem.* **1980**, *112*, 557; (**c**) Bouvier, P.; Berger, Y.; Rohmer, M.; Ourisson, G. *Eur. J. Biochem.* **1980**, *112*, 549; (**d**) Abe, I.; Rohmer, M. *J. Chem. Soc. Perkin Trans. 1* **1994**, 783.
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