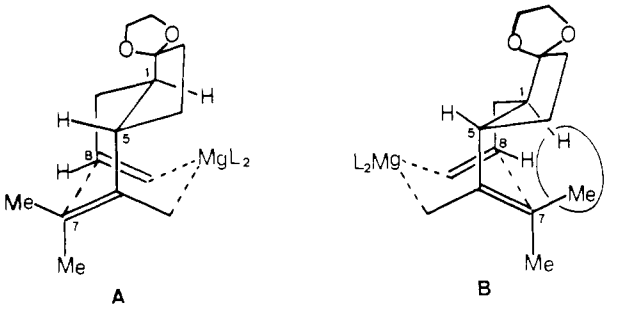
results,[7] its virtually quantitative stereoselectivity is particularly noteworthy. Assuming kinetic stereoselection the alternative transition states A and B have been examined. Indeed, B shows



a boat conformation of the developing cyclohexane, causing severe flagpole repulsion of one C(7) methyl and the C(1) hydrogen, whereas the evolving chair in A is perfectly attainable. We thus predicted A to be favored over B, which entails the desired cis disposition of H–C(5) and H–C(8) in **8**. Unambiguous evidence for this stereochemical assignment was provided by the transformation of **9** into (±)-khusimone as follows. Reduction of the carboxylic acid **9** with $LiAlH_4$, mesylation of the primary alcohol[8] (MsCl, NEt_3), and subsequent acetal cleavage (aqueous HCl, ether) furnished after crystallization the ketomesylate **10**[8] (mp 107.5–108.5 °C, ether–pentane, 86% yield from **9**). Finally, intramolecular alkylation of **10** by brief exposure to *t*-BuOK, *t*-BuOH, and C_6H_6 furnished after sublimation (70–80 °C (bath) (0.04 torr)) pure (±)-khusimone (**1**;[14] mp 72.5–73.5 °C, 98% yield), identified by comparison with authentic (–)-**1** (GC,[15] IR, ^1H NMR, ^{13}C NMR, and MS). In summary, (±)-khusimone was obtained from cyclopentenone by a sequence of nine synthetic operations in 11% overall yield. This strategic application of the remarkably regio- and stereoselective "magnesium-ene" reaction **7** → **8** exemplifies the potential value of this method in synthesis.

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Registry No. (±)-**1**, 64550-95-4; **2**, 930-30-3; **3**, 638-10-8; **4**, 106-95-6; **5**, 83291-58-1; (±)-**6**, 83291-59-2; (±)-**7**, 83291-60-5; (±)-**8**, 83291-61-9; (±)-**9**, 83291-62-7; (±)-**10**, 83291-63-8.

(14) No trace of epikhusimone was detected (^1H NMR) in the crude cyclization product.

(15) GC comparison of (±)-**1** with (–)-**1** was carried out by co-injection using a 24-m capillary column, OV 101, 220 °C.

Action of 2,3-Oxidosqualene Lanosterol Cyclase on 15′-Nor-18,19-dihydro-2,3-oxidosqualene

Eugene E. van Tamelen,* Eric J. Leopold, Stuart A. Marson, and Hans R. Waespe

Department of Chemistry, Stanford University Stanford, California 94305

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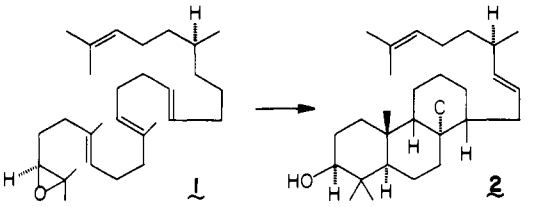
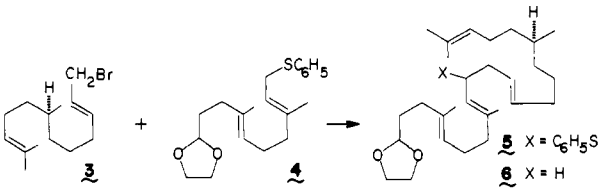
In an endeavor to probe the rigidly enzyme controlled[1] chemistry of ring C formation during lanosterol biosynthesis, the action of 2,3-oxidoxqualene lanosterol cyclase on a particular substrate



1 **2**

variant, 15′-nor-18,19-dihydro-2,3-oxidosqualene (**1**) was investigated. Results summarized herein reveal the final product to be tricycle **2**, presumably generated by hydrogen transfer from the side chain to the C ring of the evolving tricyclic intermediate.

Coupling of trans-bromide **3**[2] and trans,trans-sulfide **4**[2] con-

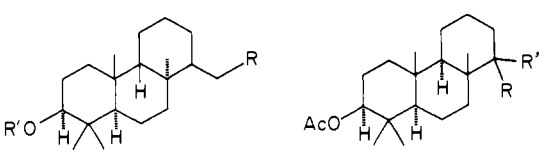


3 **4** **5** X = C_6H_5S **6** X = H

situtes the integral part of the oxide **1** synthesis, accomplished by initial conversion of **4** to its anion with *n*-C_4H_9Li followed by addition of **3** (THF, –78 °C → room temperature). The resulting polyolefinic thioether **5** (69% yield) was then subjected to the action of $Li/C_2H_5NH_2$ at -78 °C, yielding (66%) the acetal **6**. Tritium labeling was carried out by quantitative hydrolysis of the acetal (3% aqueous $HClO_4$/THF, 40 °C) to the parent aldehyde and exposure of the latter to THF/^3H_2O (1 Ci/mL) to which had been added PCl_5. On treatment with $(C_6H_5)_2SC(CH_3)_2$ (THF, –78 °C), the radiolabeled aldehyde was transformed (70%) into epoxide [4-^3H]**1**, purified by prep TLC (specific ^3H activity 6.77 × 10^4 dpm/μg).

The enzymic cyclization was carried out by means of rabbit liver cyclase, as previously described.[3] Incubation of **1** (2.20 mg, 14.9 × 10^7 dpm) at 37 °C for 60 min with a clarified (10.5 × 10^4*g* supernatant) enzyme preparation obtained from the microsomal fraction, followed by denaturization with 1 N methanolic KOH and then ether extraction, gave total product representing 88% recovery of radioactivity. Appropriate boiled controls were carried out. After prep TLC, there were isolated starting material (81%), presumed 2,3-glycol (8%), and a sterol fraction (7%: **2**, R_f 0.28; lanosterol, R_f 0.31), which was purified by HPLC (radioactivity-based percentages of total enzymic product).

High-resolution mass (M^+ 414.3833) and time-averaged 360-MHz NMR (benzene-d_6) spectra indicated that the enzymic product is a polycycle with the same elementary composition as oxide **1** and having an equatorial C-3 hydroxyl (δ 2.98–3.11), five methyls on saturated carbon (0.82–1.06), an isopropylidene unit (1.63, 1.72), and a disubstituted double bond (5.33–5.42). Hydrogenation (Pd/C, EtOAc) afforded a tetrahydro product (*m/e* 418). In order to locate the nonterminal site of unsaturation, oxidative olefin cleavage was carried out with $NaIO_4/OsO_4$ (dioxane/H_2O; 25 °C). High-resolution mass ($M^+ - H_2O$ 288.2455) and NMR spectra revealed the major cleavage product to be a $C_{20}H_{34}O_2$ aldehydro alcohol, resulting from loss of a C_9 side chain fragment. In confirmation of this assignment, $NaBH_4$



7 R = CHO, R′ = H
8 R = CH_2OAc, R′ = Ac

9 R, R′ = O
10 R, R′ = $CHCO_2Et$
11 R = H, R′ = CH_2CH_2OH

(1) van Tamelen, E. E. *Int. Congr. Pure Appl. Chem., 23rd*, **1971**, *5*, 85. van Tamelen, E. E.; Willett, J.; Schwartz, M.; Nadeau, R. *J. Am. Chem. Soc.* **1966**, *88*, 5937.

(2) Synthesis to be described elsewhere.

(3) van Tamelen, E. E.; Hopla, R. E. *J. Am. Chem. Soc.* **1979**, *101*, 6112.

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reduction (THF, 25 °C) yielded a $C_{20}H_{36}O_2$ diol, characterized as a diacetate. In the customary assumption[4] that the structure and stereochemistry within the ABC framework correspond to those resulting from the normal biosynthetic pathway, structures **2**, **7**, and **8** may be allocated to the enzymatic product from oxide **1**, the cleavage product, and the derived diacetate, respectively. A mass spectral comparison substance was synthesized from a tricycle of secure structure, the keto acetate **9**,[5] in order to support these assignments. Initial reaction with ethoxyacetylide[5b] (Et_2O, −10 to −16 °C → room temperature), followed by 5% aqueous H_2SO_4 (MeOH, room temperature) produced the α,β-unsaturated ester **10**. Lithium aluminum hydride reduction (refluxing THF) generated (in addition to the allyl alcohol) the saturated diol **11**. The (GC facilitated) mass spectrum of its diacetate was *qualitatively* virtually indistinguishable (essential peak for peak matching, but differing intensities), from that of diacetate **8**, in keeping with the skeleton and functionality of **8** and therefore structures **7** and **2**. Buttressing of these assignments is embodied in the close similarity[6] of the NMR (100 MHz, benzene-d_6) C–Me signals of **7** (δ 0.76, 2 × 0.84, 1.04) and **11** (δ 0.77, 0.81, 0.82, 0.97), especially in regard to the ones at highest field (ring-C Me's), a particular comparison rendering unlikely a conceivable **7**/**2** alternative, viz., the isomeric perhydrocyclopenta[*a*]-naphthalene, for which a cyclopentanoid methyl peak at δ ~0.69 would be expected.[7]

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(4) E.g.: Corey, E. J.; Lin, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1969**, *91*, 2132.
(5) (a) Ireland, R. E.; Baldwin, S. W.; Dawson, D. J.; Dawson, M. I.; Dolfini, J. E.; Newbould, J.; Johnson, W. S.; Brown, M.; Crawford, R. J.; Hudrlik, P. F.; Rasmussen, G. H.; Schmiegel, K. K. *Ibid.* **1970**, *92*, 5743. (b) Baldwin, S. W. Ph.D. Dissertation, California Institute of Technology, 1969.
(6) In related cases, chemical shifts of angular Me's in tricyclic ABC systems are virtually independent of trans,anti,trans and trans,syn,trans relative stereochemistry: van Tamelen, E. E.; Sharpless, K. B.; Hanzlik, R.; Clayton, R. B.; Burlingame, A. L.; Wszolek, P. C. *J. Am. Chem. Soc.* **1967**, *89*, 7150. Sharpless, K. B. Ph.D. Dissertation, Stanford University, 1968.
(7) Zücher, R. F. *Helv. Chim. Acta* **1963**, *46*, 2054 and spectra of other cyclopentanes from this laboratory.

Bioorganic Characterization and Mechanism of the 2,3-Oxidosqualene → Lanosterol Conversion

Eugene E. van Tamelen

Department of Chemistry, Stanford University
Stanford, California 94305

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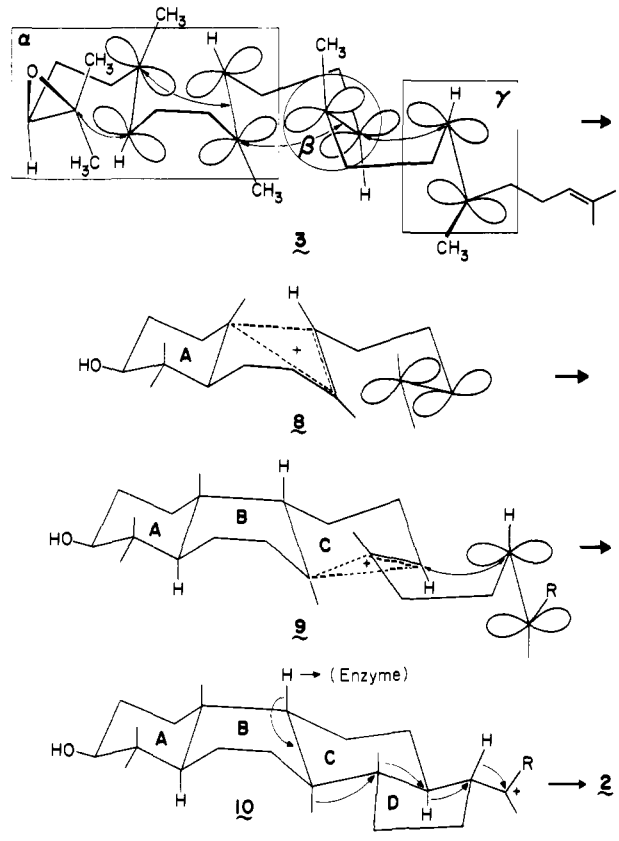
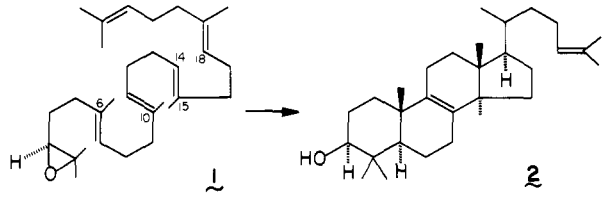
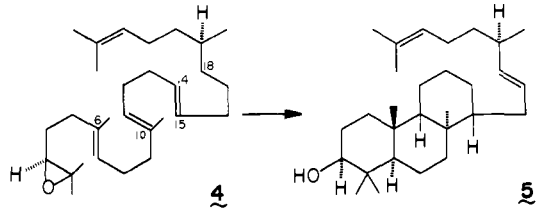
In regard to the biological conversion of 2,3-oxidosqualene (**1**) to lanosterol (**2**), previous study[1-7] of various enzymic and nonenzymic reactions of squalene oxide and its variants have led to inter alia the following observations and inferences regarding the cyclization process: (a) polycyclization involves A-ring formation with a high degree of S_N2-like participation of the neighboring, Δ^6 π bond[5] and an ensuing series of conformationally rigid, partially cyclized carbocationic intermediates;[4,5,7] (b) the oxide–tetra-π-bond sequence (α, β, γ) in **3** constitutes the essential substrate requirement for tetracyclization, the nonoxidic C-5 terminus and the methyls at C-6, -10, and -15 not being individually necessary;[1] (c) the chiral, trisubstituted oxide, Δ^6, Δ^{10} array (α) currently represents the minimum requirement for significant cyclase action;[1] (d) distances (↔, **3**) between, and required conformational orientations of, C-2 and C-7,[6] C-6 and Δ^{10} as well as C-10 and Δ^{14}, must be optimized;[1] (e) except for the terminating C-9 proton loss and for behavior in the Δ^{14} area, all chemical (including conformational) behavior can be qualitatively simulated in nonenzymic, related systems.[1-3] By contrast, illuminating biochemical information regarding relationships between the Δ^{10} and Δ^{18} sites and that at Δ^{14} (β) has been lacking, a shortcoming alleviated by the recent finding[8] that 15′-nor-18,19-dihydrosqualene 2,3-oxide (**4**) is transformed enzymically

Scheme I







(1) van Tamelen, E. E. *Int. Congr. Pure Appl. Chem., 23rd* **1971**, *5*, 85 and references cited therein.
(2) van Tamelen, E. E. *Acc. Chem. Res.* **1968**, *1*, 111; **1975**, *8*, 152.
(3) van Tamelen, E. E.; Anderson, R. J. *J. Am. Chem. Soc.* **1972**, *94*, 8225.
(4) van Tamelen, E. E.; Lees, R. G.; Grieder, A. *J. Chem. Soc.* **1974**, *96*, 2255.
(5) van Tamelen, E. E.; James, D. R. *J. Am. Chem. Soc.* **1977**, *99*, 950.
(6) van Tamelen, E. E.; Pedlar, A. D.; Li, E.; James, D. R. *J. Am. Chem. Soc.* **1977**, *99*, 6778.
(7) van Tamelen, E. E.; Hopla, R. E. *J. Am. Chem. Soc.* **1979**, *101*, 6112.
(8) van Tamelen, E. E.; Leopold, E.; Marson, S. A.; Waespe, H. R. *J. Am. Chem. Soc.*, preceding communication in this issue.

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