rin-containing macrocycles has recently been shown to also afford very large rings.[10]

Acknowledgment. We thank the CNRS and the Conseil Général de la Région Alsace for their financial supports.

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An Experimental Demonstration of the Stereochemistry of Enzymic Cyclization of 2,3-Oxidosqualene to the Protosterol System, Forerunner of Lanosterol and Cholesterol

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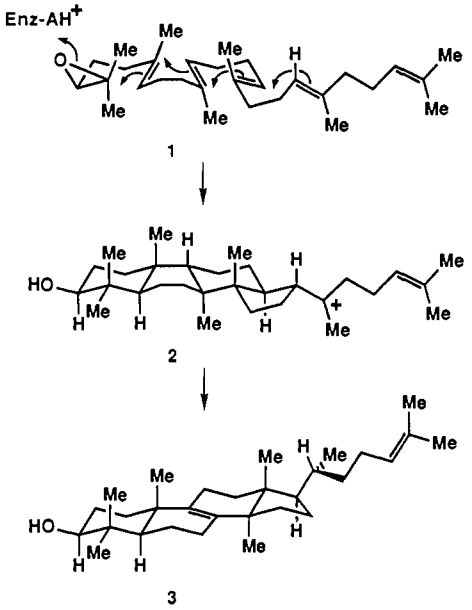
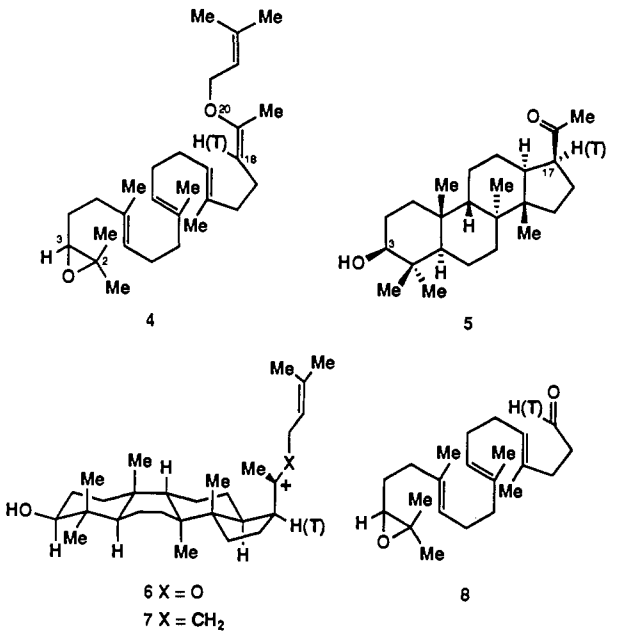
Received January 24, 1991

The biosynthesis of sterols is generally thought to be initiated by the enzymic cyclization of 2,3-oxidosqualene[1] (**1**) to the cationic protosterol **2**[2] (or equivalent), which undergoes a series of suprafacial 1,2-shifts leading, after proton loss, to lanosterol (**3**).



A problem associated with this scheme is that a rotation about the C(17)–C(20) bond of 120° is required prior to H migration from C(17) to C(20) in order to produce the natural *R* configuration at C(20), whereas only a 60° rotation (counterclockwise when viewed down the C(17)–C(20) axis) would lead, after H shift, to the unnatural *S* configuration at C(20). Perhaps because of this difficulty, it has been proposed that the initial tetracyclic intermediate is not cation **2** but an equivalent that results from covalent attachment of some nucleophile from the cyclase enzyme, often referred to as an "X group", to the *re* face of C(20) during the closure of ring D.[3,4] Reported herein is a demonstration that the closure of ring D during sterol biosynthesis produces a protosterol with a 17β-oriented side chain rather than the 17α arrangement previously assumed (**2** or its X-group equivalent).[2,3] The experimental evidence includes the bioconversion of an analogue of 2,3-oxidosqualene to a protosterol,[5] which was identified by comparison with totally synthetic material.[6]

The (±)-20-oxa analogue of 2,3-oxidosqualene (**4**) and the 18-tritio form of **4** were synthesized as described below and incubated with sterol-free microsomal protein from bakers' yeast (*Saccharomyces cerevisiae*)[7,8] at pH 6.2 (0.5 M phosphate buffer containing 0.3% Triton X-100) at 23 °C for 40 h. The product of cyclization was isolated by removal of water in vacuum, addition of tetrahydrofuran (THF), drying, evaporation, and chromatography on silica gel, in a 1:1 ether–hexane fraction. Although the principal product is chromatographically similar to ergosterol (TLC R_f 0.27, silica gel, 1:1 ether–hexane), it was not this yeast sterol, but the 17β-acetyl protostane derivative, **5** (56% of the theoretical yield).[9] Biosynthetic **5** was converted to the 3-*tert*-butyldimethylsilyl (TBS) ether (TBSCl, imidazole, DMF, 35 °C, 6 h) and compared with totally synthetic material, prepared as described below. The biosynthetic and synthetic 3-TBS ethers of **5**, mp 200–202 °C (undepressed on admixture), $[\alpha]^{23}_D$ +65.8° (c = 0.6, $CHCl_3$), were identical in all respects, including TLC mobility, 500-MHz ^1H and 125-MHz ^{13}C NMR spectra, and infrared and high-resolution mass spectra. Treatment of biosynthetic **5** TBS ether with 1% KOH in 1:1:1 THF–CH_3OH–H_2O at 50 °C for 2 h effected complete isomerization to the more stable C(17) diastereomer (17α-acetyl), which was identical with a totally synthetic standard by ^1H NMR, IR, HRMS, and TLC comparison.[10]



(1) (a) Corey, E. J.; Russey, W. E.; Ortiz de Montellano, P. R. *J. Am. Chem. Soc.* **1966**, *88*, 4750. (b) Corey, E. J.; Russey, W. E. *J. Am. Chem. Soc.* **1966**, *88*, 4751. (c) Van Tamelen, E. E.; Willet, J. D.; Clayton, R. B.; Lord, K. E. *J. Am. Chem. Soc.* **1966**, *88*, 4752–4753.

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(5) For early examples of biosynthetic protosterols, see: (a) Corey, E. J.; Ortiz de Montellano, P. R.; Yamamoto, H. *J. Am. Chem. Soc.* **1968**, *90*, 6254–6255. (b) Corey, E. J.; Lin, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1969**, *91*, 2132–2134.

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(7) (a) Caras, I. W.; Bloch, K. *J. Biol. Chem.* **1979**, *254*, 11816–11821. (b) Medina, J. C.; Kyler, K. S. *J. Am. Chem. Soc.* **1988**, *110*, 4818–4821.

(8) Type II bakers' yeast (Sigma) was washed and suspended in pH 7 phosphate buffer, lysed by two passages through a French pressure cell, and centrifuged at 10000*g* to afford a supernatant, which was centrifuged at 100 000 g. The microsomal pellet was resuspended in pH 6.2, 0.025 M phosphate buffer containing 2% Triton X-100 and centrifuged at 100 000 g, and the protein in the supernatant was freed of sterols by using a hydroxylapatite column.

(9) In contrast the 18,19(*Z*)-isomer of **4** is not converted to sterol products by the cyclase under the above-described conditions (which afford a >70% yield of lanosterol from 2,3-oxidosqualene).

(10) During the 17β → 17α epimerization, radioactivity was removed from the 17-tritiated TBS ether of **5** produced biosynthetically from 18-tritiated **4**.

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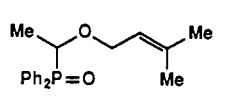
The stereospecific enzymic conversion of **4** to **5** implies the intermediacy of cation **6** for this cyclization and suggests an analogous intermediate (**7**) for the enzymic conversion of 2,3-oxidosqualene to lanosterol. This finding removes the need to invoke covalent binding of C(20) to the cyclase in lanosterol biosynthesis from 2,3-oxidosqualene, since the initially formed conformation of the protosterol C(20) cation can lead to the natural C(20) configuration via a least motion pathway involving only a small (<60°) rotation about the C(17)-C(20) axis.



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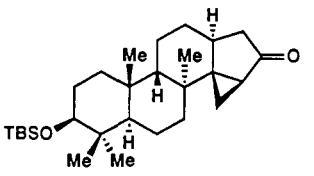

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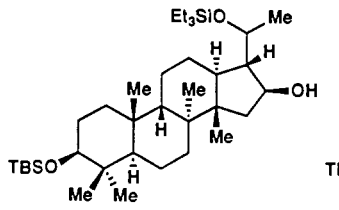

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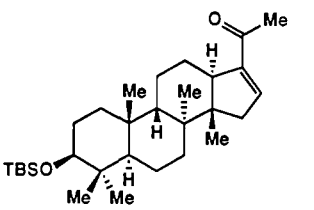

12

Substrate **4** was synthesized from epoxy aldehyde **8**[5b,11] and phosphine oxide **9**[12] by the following sequence: (1) conversion of **9** to the lithio derivative (LDA in THF at −90 °C) and reaction with the aldehyde **8** at −90 °C for 15 min to give after extractive isolation a 1.3:1 mixture (88% yield) of two diastereomeric β-hydroxy phosphine oxides; (2) chromatographic separation on silica gel–1% H_2O using ether–H_2O (100:1) to elute the minor diastereomer, R_f 0.51 (ether), and the major diastereomer, R_f 0.35 (ether); (3) reaction of the major diastereomer with NaH–NaOH–THF at 23 °C for 24 h to form **4** stereoselectively in 93% yield.[13] Radiolabeled **4** containing tritium at C(18) was synthesized from 1-tritiated aldehyde **8**.

The enzymic cyclization product **5** was synthesized as the 3-TBS ether from the previously synthesized protosterol precursor **10**.[6] Ketone **10** was converted to the triol derivative **11** by existing methodology[6] using acetaldehyde as an aldol component. Intermediate **11** was transformed into enone **12** in 88% overall yield by the following sequence: (1) mesylation ($MeSO_2Cl$, Et_3N, CH_2Cl_2, 0 °C, 1h); (2) cleavage of triethylsilyl (1% CF_3CO_2H in THF–H_2O, 23 °C, 6 h); (3) oxidation (pyridinium chlorochromate–Al_2O_3, CH_2Cl_2, 23 °C, 6 h); and (4) elimination (KO-*t*-Bu–THF, 23 °C, 3 h). Reduction of enone **12** by 4 equiv of lithium in 1:1 THF–liquid NH_3 containing 2 equiv of H_2O[6] at −35 °C for 2 min afforded the 3-TBS ether of **5** (30%) and the less polar 17α-epimer (52%, mp 190–192 °C).[14]

In conclusion, this work has revealed that the enzymic conversion of 2,3-oxidosqualene (**1**) to lanosterol proceeds by the cyclization of **1** to a protosterol intermediate having a β-oriented side chain at C(17), as in **7**. The long-held alternative **2**[2] is untenable, and it is unnecessary to postulate covalent attachment[3] of the intermediate protosterol to the cyclase enzyme.[15]

Supplementary Material Available: Syntheses of **8** and **4** from squalene, enzymic preparation of **5** and identification of its *tert*-butyldimethylsilyl ether derivative, and preparation of totally synthetic *tert*-butyldimethylsilyl ether derivatives of **5** and 17α-acetylprotosterol (21 pages). Ordering information is given on any current masthead page.

(11) A convenient large-scale synthesis of **8** is described in the supplementary material.

(12) (a) The phosphine oxide **9** was synthesized by the following sequence: (1) reaction of 3-methyl-2-butenyl vinyl ether (Boeckman, R. K., Jr.; Ko, S. S. *J. Am. Chem. Soc.* **1982**, *104*, 1033–1041) with triphenylphosphonium bromide in THF at 23 °C and (2) treatment with aqueous NaOH at 45 °C (see: Ley, S. V.; Lygo, B.; Organ, H. M.; Wonnacott, A. *Tetrahedron* **1985**, *41*, 3825–3836). (b) See also: Ceruti, M.; Viola, F.; Dosio, F.; Cattel, L.; Bouvier-Navé, P.; Ugliengo, P. *J. Chem. Soc., Perkin Trans. 1* **1988**, 461–469.

(13) The *Z* isomer of **4** was similarly obtained from the minor diastereomeric β-hydroxy phosphine oxide. The stereochemistries of **4** and the *Z* isomer were assigned from NOE experiments.

(14) The R_f values for **5** and the 17-epimer determined by using silica gel with 1:3 ether–hexane were 0.53 and 0.49, respectively. The configuration at C(17) of **5** follows from the quantitative base-catalyzed conversion to the 17-epimer and also from X-ray diffraction data, to be published separately.

(15) We are indebted to Mr. Seiichi P. T. Matsuda for his assitance in the preparation of microsomal cyclase and to Prof. Ian Scott of Texas A and M University for information on the stability of the yeast cyclase as a function of pH. This research was assisted financially by a grant from the National Institutes of Health and an NSF graduate fellowship to S.C.V.

Highly Enantioselective and Diastereoselective Ireland–Claisen Rearrangement of Achiral Allylic Esters

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Received February 19, 1991

The Ireland–Claisen rearrangement of allylic esters, as silyl enol ethers,[1] has found broad application in synthesis especially because of its diastereoselectivity when two adjacent stereocenters are produced. Reported here is the first enantioselective version of this process with achiral esters, which depends on a readily available and recyclable chiral boron reagent.[2] A number of other approaches to enantioselective aza-Claisen and acetal Claisen rearrangements, mainly using chiral substrates, have been described recently.[3-5]

The chiral bromoborane **1** (or its enantiomer) has been employed previously for the conversion of propionate esters to either of the isomeric enolates.[2a] In a similar way, the reagent **1** can be used to convert (*E*)-crotyl propionate (**2**) to either the (*E*,*E*)-enolate (**3**) or the (*E*,*Z*)-enolate (**4**) simply by a change of the solvent and the tertiary amine used for enolate formation (Scheme I). The (*E*)-enolate **3** [formed in 24 h at −78 °C in CH_2Cl_2 with $(i\text{-Pr})_2NEt$ as base] and the (*Z*)-enolate **4** (formed in 24 h at −78 °C in 1:2 toluene–hexane with Et_3N as base) undergo Claisen rearrangement upon storage at −20 °C for 14 days to afford Claisen rearrangement products in good yield after aqueous workup, along with the recovered bis-sulfonamide precursor of bromoborane **1**, which was efficiently recovered for reuse. The reaction in CH_2Cl_2 via **3** produced the threo acid **5** (75% yield) of >97% ee and with 99:1 threo–erythro selectivity, whereas the reaction in toluene–hexane via **4** gave the erythro acid **6** (65%) in 96% ee and with 90:10 erythro–threo selectivity.[6]

The scope of this enantioselective Claisen rearrangement was evaluated by the study of the examples summarized in Tables I and II. The Claisen rearrangements of various allylic propionate and butyrate esters generally proceed with remarkably high enantioselectivity, and in many cases the analytical method could not detect enantiomeric contamination. Lower enantioselectivities

(1) Ireland, R. E.; Mueller, R. H.; Willard, A. K. *J. Am. Chem. Soc.* **1976**, *98*, 2868–2877.

(2) (a) Corey, E. J.; Kim, S. S. *J. Am. Chem. Soc.* **1990**, *112*, 4976–4977. (b) Corey, E. J.; Imwinkelried, R.; Pikul, S.; Xiang, X. B. *J. Am. Chem. Soc.* **1989**, *111*, 5493–5495.

(3) Aza-Claisen rearrangement: (a) Kurth, M. J.; Decker, O. H. W.; Hope, H.; Yanuck, M. D. *J. Am. Chem. Soc.* **1985**, *107*, 443–448. (b) Bailey, P. D.; Harrison, M. J. *Tetrahedron Lett.* **1989**, *30*, 5341–5344.

(4) Phosphonate anion Claisen rearrangement: Denmark, S. E.; Marlin, J. E. *J. Org. Chem.* **1987**, *52*, 5742–5745.

(5) Catalytic Claisen silyl vinyl ether rearrangement: Maruoka, K.; Banno, H.; Yamamoto, H. *J. Am. Chem. Soc.* **1990**, *112*, 7791–7793.

(6) The determination of the ratio of erythro–threo diastereomers was made by GC analysis of the benzyl esters. The absolute configuration of **5** was determined by conversion to (*S*,*S*)-(−)-2,3-dimethylsuccinic anhydride; see: Berner, E.; Leonardsen, R. *Justus Liebigs Ann. Chem.* **1939**, *538*, 1–43. The absolute configuration of **6** is assigned by analogy with similar examples which follow.

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