Large crystals of **2** slowly appeared over the period of several days after irradiation. The product was harvested in 50% yield and characterized by infrared spectroscopy as well as by a single-crystal X-ray study. This observation is consistent with excitation of the $\delta \rightarrow \delta^*$ transition for an unbridged solution isomer of **1** with subsequent rotation about the Mo–Mo bond to give **2**. For samples of **1** in CH_3CN, the $\delta \rightarrow \delta^*$ transition occurs as a broad feature at λ = 520 nm. Presumably the concentration of unbridged isomers of **1** can be increased by photochemical or thermal dissociation of Mo–O bridge bonds, and indeed, solutions of **1** in acetone or CH_3CN that have been subjected to light from an intense white light source or heated to reflux also yield large quantities of isomer **2**. Acetonitrile solutions of **1** stored in the dark give only trace quantities of **2** upon workup. These findings point to a subtle difference in energies between two isomeric forms of $Mo_2(O_2CCF_3)_4(bpy)_2$ with preference for the neutral complex occurring in less polar solvents and under conditions that increase the likelihood of forming unbridged isomers. The high-temperature isomerization of **1** to **2** suggests that the unsupported structure is actually more thermodynamically stable than the cis-trifluoroacetate-bridged structure. Although this conclusion may appear to be counterintuitive, it is not unreasonable if one compares the bonding interactions in the two structures. Molecule **2** possesses a much shorter Mo–Mo bond and stronger Mo–O interactions than **1**. Additional work on this intriguing system is in progress.

Acknowledgment. We are grateful to the National Science Foundation for instrumentation grants (X-ray CHE-8908088 and NMR CHE-8800770) and grants to K.R.D. (CHE-8914915) and G.C. (CHE-8808019). We also thank Professor D. G. Nocera and Carolyn Hsu for use of the photochemical equipment and Steven C. Haefner for helpful discussions.

Supplementary Material Available: Tables and summaries of X-ray data, positional parameters, bond distances, bond angles, and thermal parameters for **1** and **2** (18 pages); tables of observed and calculated structure factors for **1** and **2** (45 pages). Ordering information is given on any current masthead page.

New Mechanistic and Stereochemical Insights on the Biosynthesis of Sterols from 2,3-Oxidosqualene

E. J. Corey,* Scott C. Virgil, and Sepehr Sarshar

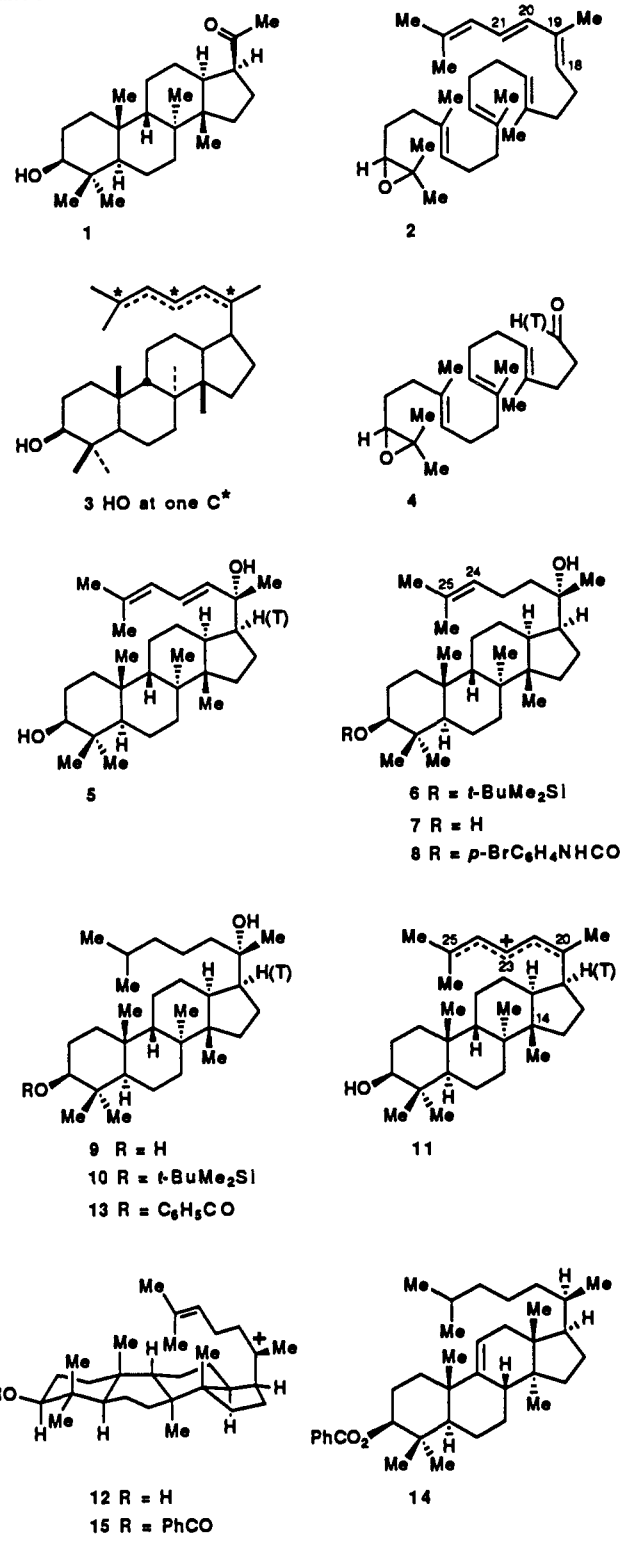
Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138

Received May 20, 1991

The enzymic cyclization of 2,3-oxidosqualene to lanosterol is of great interest from both chemical and biochemical perspectives. The recent demonstration that the 20-oxa analogue of 2,3-oxidosqualene is converted by the cyclase from yeast to the 17β-acetyl sterol **1**[1] has provided new information on the stereochemical course of the cyclization and has suggested a more detailed analysis of the previously reported bioconversion of (20*E*)-20,21-dehydro-2,3-oxidosqualene (**2**) to a protosterol of gross structure **3**[2] (Chart I). We report herein on the complete structure of **3** and its bearing on the detailed mechanism of action of the cyclase.

Tritiated (±)-**2** was synthesized from epoxy aldehyde **4**[1] by Wittig coupling[2] (7:3 mixture of 18*E* and 18*Z* isomers, separable by HPLC). Biosynthetic experiments were carried out using sterol-free microsomal enzyme of *Saccharomyces cerevisiae* (yeast) which had been purified by successive chromatography on DEAE and hydroxylapatite columns.[3] The structures of **2** (more polar isomer) and the less polar 18*Z* isomer were clear from ^1H NMR NOEDIFF measurements at 500 MHz. Whereas the 18*Z* isomer of **2** was not transformed into sterol by the cyclase, **2** was converted in ca. 30% yield (HPLC analysis) to a protostanediol, which was demonstrated to be **5** by 500-MHz ^1H NMR analysis and comparison with synthetic compounds as described below.[4] Incubation of **2** with a cyclase-containing homogenate of porcine liver[2] at 23

Chart I



(1) Corey, E. J.; Virgil, S. C. *J. Am. Chem. Soc.* **1991**, *113*, 4025–4026.
(2) Corey, E. J.; Lin, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1969**, *91*, 2132–4.
(3) Corey, E. J.; Matsuda, S. P. T. *J. Am. Chem. Soc.*, preceding paper in this issue.
(4) Preparative-scale enzymic experiments were performed using sterol-free enzyme from yeast at 23 °C for 24 h. After chromatographic purification, pure **5** was isolated in 17% yield.

0002-7863/91/1513-8171$02.50/0

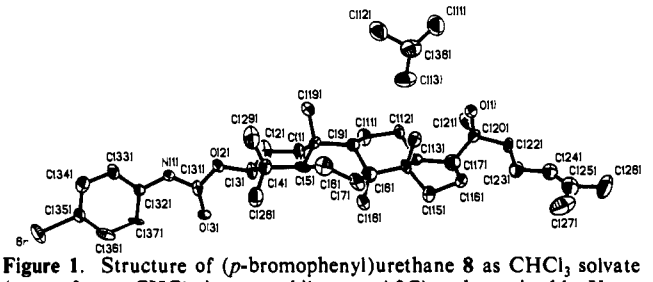


Figure 1. Structure of (*p*-bromophenyl)urethane **8** as $CHCl_3$ solvate (grown from a $CHCl_3$–isooctane bilayer at 4 °C) as determined by X-ray diffraction. Selected distances (Å): C(20)–C(21) = 1.527; C(20)–O(1) = 1.444.

°C and pH 7 for 12 h gave, after chromatographic purification, the same product (**5**) in 14% isolated yield (0.35 mg).

Reaction of the 3-*tert*-butyldimethylsilyl ether of **1**, which is available from either total synthesis[2,5] or biosynthesis,[2] with the Grignard reagent $Me_2C{=}CHCH_2CH_2MgBr$ in THF at 0 °C for 3 h produced **6** with 9:1 diastereopreference over the more polar C(20) diastereomer.[6] Conversely, the C(20) diastereomer of **6** was the major product (ca. 9:1 diastereoselection) of the reaction of CH_3MgBr with the ketone corresponding to the replacement of CH_3CO in **1** by $(CH_3)_2C{=}CHCH_2CH_2CO$. The proof of structure of **6** was effected by desilylation to **7** and conversion to the (*p*-bromophenyl)urethane **8**, whose structure was determined by single-crystal X-ray diffraction analysis to be as shown in Figure 1.[7] Diols **5** and **7** were interrelated by conversion to identical samples of diol **9**[8] and silyl ether **10**.[8]

The finding that **2** is converted to the protosterol derivative **5** by the sterol cyclases has several mechanistic implications: (1) Closure of the D ring generates a protosterol having the 17β side chain.[1] (2) Water attaches preferentially to C(20) of the tetracyclic C(20)–C(25) pentadienyl cation from **2** (**11**) at a rate which is fast compared to rearrangement of hydrogen from C(17) to C(20).[9] (3) In the biocyclization of **2** to form **5** the addition of carbon and HO to the C(18)–C(19) double bond of **2** is stereospecific and antarafacial, implying that the attachment of water to C(20) in **11** is fast relative to C(17)–C(20) bond rotation. (4) The position-specific attachment of water to cation **11** at C(20) suggests that C(23) and C(25) may be in a binding pocket which shields them from nucleophilic attack. (5) The enzyme-associated water which converts **11** to **5** may stabilize the protosterol C(20) cation from 2,3-oxidosqualene (**12**), but does not react with it at a rate which is appreciable relative to hydride migration from C(17) to C(20).

The β-orientation of the side chain at C(17) and the strong steric interaction with the cis 14β-methyl substituent clearly serve to hinder rotation about the C(17)–C(20) bond in **11** and thereby favor stereospecific attachment of hydroxyl to C(20). The β-orientation of the side chain at C(17) also facilitates the control of configuration at C(20) in lanosterol biosynthesis from 2,3-oxidosqualene, since the protosterol cation **12** is generated in the correct geometry for C(17) → C(20) hydride migration and since C(17)–C(20) bond rotation is restricted. Dramatic support for this idea has been obtained from experiments on the chemical rearrangement of **13**, the 24,25-dihydro-3-benzoate of **7**, and the C(20) diastereomer of **13**. Reaction of **13** with BF_3 in CH_2Cl_2 at −90 °C for 3 min produced 24,25-dihydroparkeol benzoate (**14**) (90%) stereoselectively, and similarly, the C(20) epimer of **13** gave the C(20) epimer of **14** (90%). In these rearrangements there is overall retention of configuration in the replacement of the C(20) hydroxyl by hydrogen. The reaction of **13** is considered to occur by (1) complexation of BF_3 with the (thermodynamically less stable) conformation shown in formula **13**, (2) heterolysis to **15**, and (3) rearrangement of hydrogen from C(17) to C(20) at a rate which is fast compared to the restricted rotation of the C(17)–C(20) bond.[10] In contrast, as noted earlier,[5] the BF_3-catalyzed rearrangements of the 17α-epimer of **13** and its C(20)-epimer are nonstereoselective at C(20), each giving a 1:1 mixture of C(20) epimers of dihydroparkeol benzoate.[5] It is clear that in the enzymic cyclization of 2,3-oxidosqualene the 17β protosterol pathway via cation **12** is superior to the 17α protosterol pathway with respect to stereospecific production of the natural 20*R* configuration of sterols.[1,11]

Supplementary Material Available: Experimental procedures and spectroscopic data for compounds **2**, **5–10**, **13**, 20-*epi*-**6**, 20-*epi*-**9**, **14**, and 20-*epi*-**14** and a listing of crystal data, atomic coordinates, bond distances and angles, and thermal parameters for (*p*-bromophenyl)urethane **8** (35 pages); listing of observed and calculated structure factors for **8** (29 pages). Ordering information is given on any current masthead page.

(5) Corey, E. J.; Virgil, S. C. *J. Am. Chem. Soc.* **1990**, *112*, 6429–31.

(6) R_f values for **6** and the C(20) diastereomer on silica gel plates (10% ether–hexane) were 0.29 and 0.25, respectively.

(7) The conformation about the C(17)–C(20) bond of **8** as shown in Figure 1 corresponds to that which is expected to be most stable.

(8) As shown by chromatographic and spectroscopic comparison.

(9) The stabilization of the pentadienyl cation subunit in **11** relative to a localized C(20) cation probably diminishes the rate of H migration from C(17) to C(20) by several orders of magnitude.

(10) Although the conformation shown in Figure 1 is clearly more stable than the C(17)–C(20) rotamer shown in **13**, the hydroxyl group at C(20) is much more accessible sterically in **13**.

(11) This research was assisted by grants from the National Institutes of Health and the National Science Foundation. We are indebted to Mr. Seiichi P. T. Matsuda for valuable information and help on the purification of the yeast cyclase.

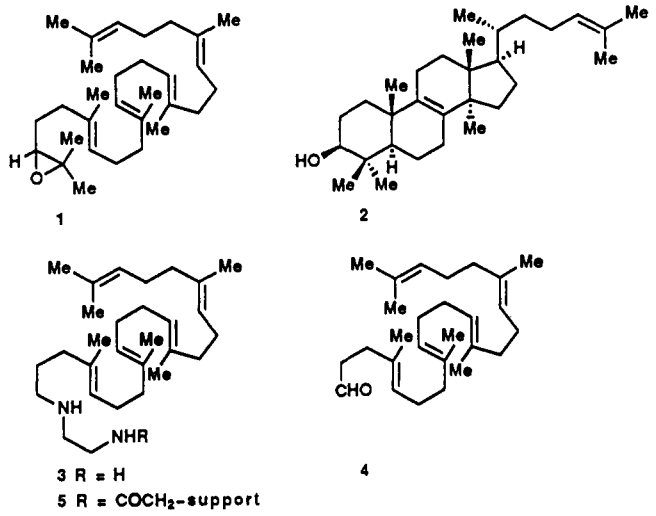
Purification of the 2,3-Oxidosqualene-lanosterol Cyclase from *Saccharomyces cerevisiae*

E. J. Corey* and Seiichi P. T. Matsuda

Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138

Received May 24, 1991

Probably the most remarkable step in the biosynthesis of cholesterol is the conversion of 2,3-oxidosqualene (**1**) to lanosterol (**2**) in a single supremely effective step by the enzyme 2,3-oxidosqualene-lanosterol cyclase EC 5.4.99.7 ("sterol cyclase").[1]



1 **2**

3 R = H
5 R = $COCH_2$-support

4

In contrast, a sequence of 18 additional steps is required to remove the three extraneous angular methyl groups of **2** and to generate

(1) (a) Corey, E. J.; Russey, W. E.; Ortiz de Montellano, P. R. *J. Am. Chem. Soc.* **1966**, *88*, 4750–4751. (b) Corey, E. J.; Russey, W. E. *J. Am. Chem. Soc.* **1966**, *88*, 4751–4752. (c) Van Tamelen, E. E.; Willet, J. D.; Clayton, R. B.; Lord, K. E. *J. Am. Chem. Soc.* **1966**, *88*, 4752–4754. (d) Eschenmoser, A.; Ruzicka, L.; Jeger, O.; Arigoni, D. *Helv. Chim. Acta* **1955**, *38*, 1890–1904.

0002-7863/91/1513-8172$02.50/0