trolled proton abstraction from C-9. In that case, the methyl–hydrogen migration sequence from a "protolanosterol" may be coordinated with, and assisted by, operation at C-9 of a specific basic enzyme center.

Acknowledgment. The authors are grateful to Dr. J. R. Trudell, Stanford Medical Center, for mass spectral determination and to the National Science Foundation for financial support (GP 7187). One of us (J. W. Murphy) thanks the Institute of Organic Chemistry, Syntex Research, Palo Alto, Calif., for partial support during this work.

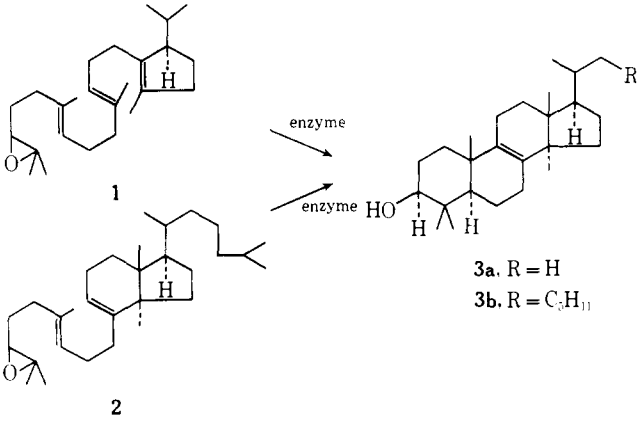
* Address correspondence to this author.

E. E. van Tamelen,* J. W. Murphy
Department of Chemistry, Stanford University
Stanford, California 94305
Received August 31, 1970

Biochemical Conversion of Partially Cyclized Squalene 2,3-Oxide Types to the Lanosterol System. Views on the Normal Enzymic Cyclization Process

Sir:

In accompanying communications,[1,2] there are described the *nonenzymic* conversions: (1) monocarbocyclic, squalene oxide like modification **1** to the tetracyclic, isoeuphenol system and (2) bicarbocyclic epoxide **2** to dihydro-9β-Δ^7-lanosterol. In addition, we have discovered that epoxides **1** and **2**—despite



being notably different in structure from the normal lanosterol biological precursor, squalene oxide—are transformed *enzymically* to pentanorlanosterol **3a** and dihydrolanosterol **3b**, respectively, without formation of detectable amounts of the aforementioned nonenzymic products.

Radiolabeled (^{3}H at C-4) substrate **1** (7.52 mg, 9.32×10^5 dpm) was incubated for 1 hr at 37° with 75 ml of cyclase preparation.[3,4] The "sterol" component isolated by silica gel tlc using ethyl acetate–hexane (20:80) was acetylated and rechromatographed using the same system. Material with an R_f corresponding to that of dihydrolanosteryl acetate (0.44–0.52; 10% ethyl acetate–hexane) was further purified by glpc (XE-60 at 180°) and used in aliquots (7.25×10^5 dpm, 6.2 μg) in all subsequent experiments. Smaller scale incubations, carried out in duplicate with ^{3}H-labeled epoxide **1**, squalene 2,3-oxide, and pentanorsqualene 2,3-oxide, using both active and denatured cyclase, showed that: (1) epoxide **1** was converted to pentanorlanosterol **3a** in an average 1.8% yield and (2) the yield of **3a** from acyclic epoxide was 2 times that from monocarbocyclic epoxide **1**, all under conditions where lanosterol was formed from squalene oxide in 56% yield.

A sample of the sterol acetate (6.82×10^4 dpm) from epoxide **1** possessed a glpc peak indistinguishable from that of 23,24,25,26,27-pentanorlanosterol. The free sterol (6.26×10^4 dpm) was converted (trimethylsilyl chloride–pyridine) to the trimethylsilyl ether (TMSE) and analyzed by glpc on DEGS at 190°. A single radioactive peak was obtained, which coinjected exactly with that of authentic 23,24,25,26,27-pentanorlansterol–TMSE (R_c = 0.77)[5] and contained 93% of the recovered radioactivity.

To 18.0 mg of authentic pentanorlanosteryl acetate was added acetylated enzyme product (2.11×10^5 dpm) and the mixture was recrystallized several times from acetone containing a trace of dichloromethane. Specific activities observed in successive crystallizations were (9.09, 8.65, 8.69, 8.61, 8.68) $\times 10^3$ dpm/mg. The mass spectrum of the acetylated enzyme product was identical with that of authentic pentanorlanosterol acetate, showing major peaks at *m/e* 400 (M^+), 385, 340, 326, 325 (base peak), 95, 81, 69, 55, and 41.

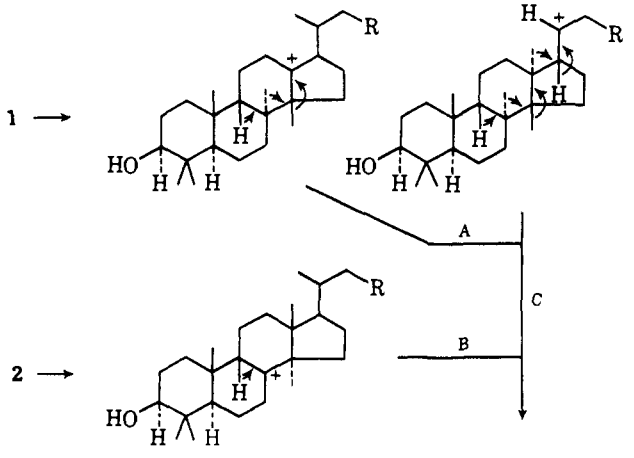
By similar means 4.29 mg (2.17×10^8 dpm) of bicarbocyclic epoxide (**2**)[4] was incubated and the resulting sterol isolated, purified, and studied. Final glpc fractionation was carried out at 210° (XE-60), and sterol acetate (7.96×10^4 dpm), which possessed the retention time expected for dihydrolanosteryl acetate (R = 11.7 min), was used in characterization experiments. In analytical runs, the average conversion was *ca.* one-half that of epoxide **1** to **3a**.

Trimethylsilyl ether secured as described in the C_{25} series was analyzed by glpc on DEGS at 200°. The single radio peak observed coinjected exactly with dihydrolanosterol–TMSE (R_c = 2.28). Similarly, cocrystallization (acetone) experiments involving an aliquot (1.95×10^4 dpm) of radioacetate and 27.9 mg of authentic dihydrolanosteryl acetate revealed the successive specific activities (5.85, 5.85, 5.80, 6.11, and 5.82) $\times 10^2$ dpm/mg. The mass spectrum of enzymic sterol acetate was identical in all respects with that of authentic dihydrolanosteryl acetate.

Despite the production of the natural product system, lanosterol, in the above experiments, the substrate epoxides **1** and **2** cannot—in view of the lack of incorporation of deuterium from D_2O during sterol biosynthesis[6]—represent true intermediates in the squalene → sterol bioconversion. Rather, the present results apparently reflect the near insensitivity of cyclase to the potential ring D area of squalene oxide types, a characteristic observed previously.[3] On the

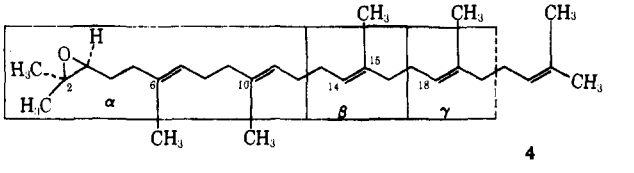
(1) E. E. van Tamelen, G. M. Milne, M. I. Suffness, M. C. Rudler, R. J. Anderson, and R. S. Achini, *J. Amer. Chem. Soc.*, **92**, 7202 (1970).
(2) E. E. van Tamelen and J. W. Murphy, *ibid.*, **92**, 7204 (1970).
(3) See for example E. E. van Tamelen, K. B. Sharpless, R. P. Hanzlik, R. B. Clayton, A. L. Burlingame, and P. C. Wszolek, *ibid.*, **89**, 7150 (1967).
(4) Radiolabeled epoxides 1 and 2 were prepared, with the assistance of Dr. G. M. Milne and Mr. J. W. Murphy, by ^3H_2O exchange of aldehyde used for conversion to epoxide with diphenylsulfonium isopropylide.[1,2]
(5) R. J. Anderson, R. P. Hanzlik, K. B. Sharpless, E. E. van Tamelen, and R. B. Clayton, *Chem. Commun.*, 53 (1969).
(6) T. T. Tchen and K. Bloch, *J. Amer. Chem. Soc.*, **78**, 1516 (1956).

other hand, it is remarkable that cyclase action is observed in substrate cases featuring three (1)[8] or as few as two (2) olefinic links. Again, the presumed predilection for 9,10 cis (B boat) intermediate formation[7] is apparent in these cases. Furthermore, the described results indicate that partially cyclized substrate can, in the process of further enzymic cyclization, merge into (A, B) the normal methyl–hydrogen migration sequence (C) which occurs subsequent to formation of tetracycle, and proceed to the lanosterol system. These results therefore lend support to the previous suggestions[8] that the methyl–hydrogen migration sequence rests solidly on organic chemical foundations and involves behavior which can be rationalized by purely chemical means. Finally, that dihydro-9-β-Δ⁷-lanosterol is produced nonenzymically from 2,[2] while the Δ⁸ isomer is generated in the enzyme



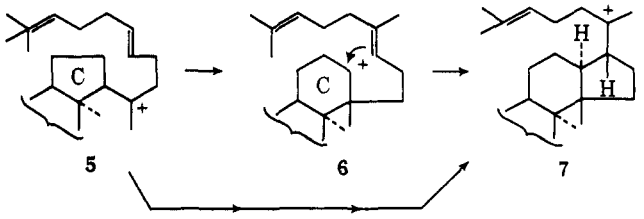
reaction, suggests that the terminating removal of the C-9 proton may be controlled by a specific basic center in the enzyme, ensuring formation of Δ⁸-lanosterol. It is pertinent that a plant cyclase produces euphol (Δ⁸) and not isoeuphol (Δ¹³⁽¹⁷⁾), the overwhelmingly more stable product.

The above results, taken together with other findings,[8] indicate that, although the trisubstituted epoxide moiety is critical, individual methyls at 6,[9] 10, and 15, and π bonds at 14 and 18, are not essential for enzymic cyclization. These reactivity patterns suggest that the epoxide–tetra-π-bond sequence (α,β,γ) (4) constitutes



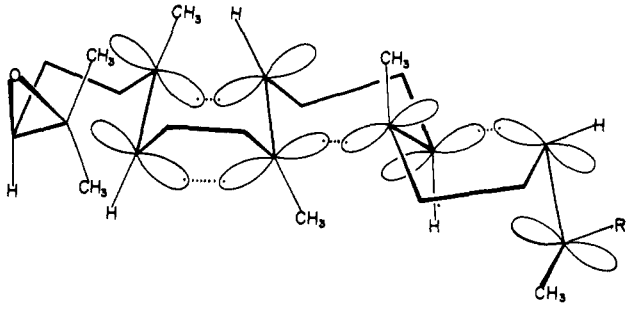
4

the essential substrate structural requirement for sterol formation and that the epoxide–bis-π-bond moiety (α) currently represents the minimum requirement for cyclase action. In addition, special enzyme control (β) is needed at the Δ¹⁴ site in order to direct carbonium ion behavior and thus realize formation of a six-membered C ring. Cyclization may proceed "nonstop" to tetracycle[7,10] or may lead to discrete carbonium ion (or derivative thereof) intermediates, *e.g.*, the chemically preferred tricycle 5. In the latter



case, conversion to sterol skeleton[7] could proceed *via* chemically precedented ring expansion (5 → 6) to six-membered C ring or by more indirect means (5 → → 7), previously considered.[11]

In terms of an extended cyclization to the protolanosterol system, the π-orbital interactions depicted in 8 would obtain; and an important function of the



8

cyclase enzyme would be maintenance of the epoxide–π system in such a conformation as to maximize the orbital overlap permitting ultimate generation of the σ-bonding system of product sterol.[11] In this stereoelectronic interpretation, three types of π interactions can be discerned: (1) epoxide-Δ⁶, permitting SN2 type attack of the π electrons on C-2, (2) Δ⁶–Δ¹⁰, in which (because of the incipient B boat conformation) full overlap of nodal extremities is realized, and (3) Δ¹⁰–Δ¹⁴ and Δ¹⁴–Δ¹⁸, distinguished by perpendicular orientation of the π planes. The implications of the difference between interactions 2 and 3 will be discussed elsewhere.

Acknowledgment. The authors wish to acknowledge financial aid from the National Institutes of Health (GM 10421) and mass spectral contributions made by Dr. J. R. Trudell, Stanford Medical Center.

(7) A. Eschenmoser, L. Ruzicka, O. Jeger, and D. Arigoni, *Helv. Chim. Acta*, **38**, 1890 (1955).
(8) E. E. van Tamelen, R. P. Hanzlik, R. B. Clayton, and A. L. Burlingame, *J. Amer. Chem. Soc.*, **92**, 2137 (1970), and references cited therein.
(9) Unpublished results, J. A. Smaal, Stanford University.
(10) G. Stork and A. W. Burgstahler, *J. Amer. Chem. Soc.*, **77**, 5068 (1955).
(11) E. E. van Tamelen, J. D. Willett, and R. B. Clayton, *ibid.*, **89**, 3371 (1967).

* Address correspondence to this author.

E. E. van Tamelen,* J. H. Freed
Department of Chemistry, Stanford University
Stanford, California 94305
Received August 31, 1970

Synthesis of Aromatic Hydrocarbons *via* Intermediate Iron Complexes[1]

Sir:

In 1956 Fischer and Böttcher reported the synthesis of certain bisareneiron(II) salts.[2,3] Except for brief

(1) Presented in part at the 159th National Meeting of the American Chemical Society, Houston, Tex., Feb 1970, Abstract ORGN-77.
(2) E. O. Fischer and R. Böttcher, *Chem. Ber.*, **89**, 2397 (1956).