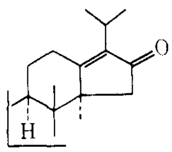
Oxidation of acetate **12**, carried out with RuO_4 or with OsO_4 followed by $NaIO_4$,[12] gave 1,5-diketone **14**[14] in high yield. Heating with KOH in diethylene glycol gave the tricyclic retro-Michael product shown to be identical (except for optical activity) with the product derived from isoeuphol (**4**)[15] by ir, mass spectral, and vpc comparisons, thereby establishing the identity of the stereochemistry of **3** with that of isoeuphol (**4**) (with the exception of the epimerizable 14-methyl). Oxidation of **12** with dipyridyl chromium trioxide gave α,β-unsaturated ketone **16**, mp 132–133° [uv (EtOH) 243 mμ (ϵ 12,600); ir ($CHCl_3$) 1725, 1685, 1630 cm^{-1}] which on ozonolysis, oxidative work-up, treatment of the acidic products with diazomethane, and chromatography gave acetoxy keto ester **17**, mp 192–194°. The ir, nmr, and mass spectra of **17** were shown to be identical with those



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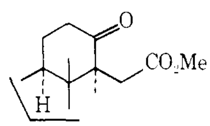

17

characteristic of the product obtained by degradation of isoeuphol.[15a] This set of results establishes without ambiguity the structure and stereochemistry of tetracycle **3** obtained by cyclization of monocyclic epoxide **1**.[16] The synthetic work herein, along with the earlier nonenzymic, selective terminal oxidation of squalene,[17] represents an overall, close simulation of the squalene → tetracyclic triterpene bioconversion and defines the purely organic chemical basis for operation of enzymes therein.

Acknowledgment. Financial support was provided by the National Science Foundation (GP 7187). The authors are also grateful to Professor D. Arigoni, Eidgenössische Technische Hochschule, for samples of euphol acetate and degradation products.

(14) Ir ($CHCl_3$) 1710 cm^{-1}; nmr ($CDCl_3$) δ 0.86 (3, s), 0.89 (6, s), 0.92 (3, s), 1.14 (3, s), 1.08 (3, d, J = 7 Hz), 1.095 (3, d, J = 7 Hz) (the isopropyl methyls are nonequivalent).

(15) (a) D. Arigoni, R. Viterbo, M. Dünnenberger, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta*, **37**, 2306 (1954). (b) For a discussion concerned with the revision of the originally assigned structure **15**, see G. V. D.-Modrone, Ph.D. Dissertation (No. 4156), Eidgenössische Technische Hochschule, Zurich, 1968.

(16) Satisfactory ir, nmr, uv, mass spectral, and analytical data were obtained for all synthetic intermediates.

(17) E. E. van Tamelen and T. J. Curphey, *Tetrahedron Lett.*, 121 (1962).

(18) NIH Postdoctoral Fellow, 1969–1970.

(19) NSF Predoctoral Fellow, 1967–1968.

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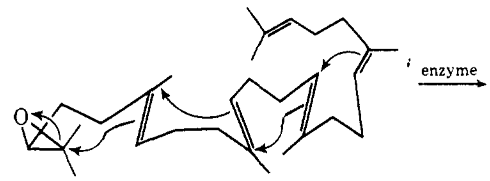
Formation of the Lanosterol System through Biogenetic-Type Cyclization

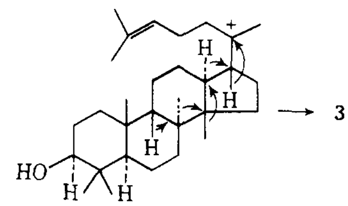
Sir:

Explicit in the Zurich proposal[1] for the biosynthesis of sterols is appearance of a "protolanosterol" intermediate (**2**) having, in addition to 8α and 14β methyls, the unusual $9\beta,10\beta$ (cis) relationship of hydrogen and methyl, a total arrangement logically permitting formation of lanosterol (**3**) structure and stereochemistry by means of a series of 1,2-methyl–hydrogen shifts and C-9 proton loss. This proposal, illustrated (Mechanism A) using the established natural substrate

Mechanism A



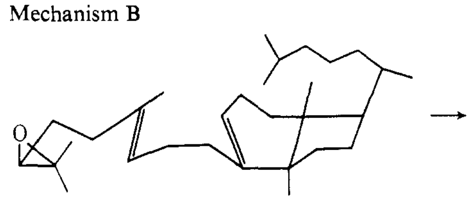
1



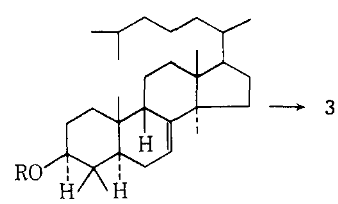
2

squalene 2,3-oxide (**1**),[2] requires the generation of a comparatively unstable ring B boat in intermediate **2**, generated by chair–boat–chair cyclization (Mechanism A). We wish to report that nonenzymic chair–boat cyclization (Mechanism B) of the polyene terminal

Mechanism B



4



5a, R = H
5b, R = CH_3CO

epoxide **4** produces a dihydrolanosterol isomer, shown to have the 9,10 cis structure **5**, which can be separately converted to Δ^8-dihydrolanosterol itself.

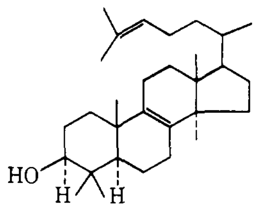
Epoxide **4** was obtained through use of a coupling reaction, the key component of which was prepared from lanosterol. Dehydrobromination (80%) of 2α-bromolanost-7-en-3-one[3] gave the Δ^1 ketone **6**: mp 123–125°; $[\alpha]^{20}D$ ($CHCl_3$) +28°; ir ($CHCl_3$) 1650 cm^{-1}; λ_{max}^{EtOH} 229 nm (ϵ 10,000); nmr (CCl_4) δ 5.30 (m, 7 H), 5.73 and 6.78 (dd, J = 10 Hz, 2 H and 1 H, respectively).

The desired cleavage of dienone **6** was effected by heating to 230–250° *in vacuo*, so that it distilled through a quartz column packed with glass helices and maintained at 600°.[4] The product was collected in a cooled

(1) A. Eschenmoser, L. Ruzicka, O. Jeger, and D. Arigoni, *Helv. Chim. Acta*, **38**, 1890 (1955).

(2) E. J. Corey and W. E. Russey, *J. Amer. Chem. Soc.*, **88**, 4750 (1966); E. E. van Tamelen, J. D. Willett, R. B. Clayton, and K. E. Lord, *ibid.*, **88**, 4752 (1966).

(3) Carried out according to the method used in the lanost-8-en-3-one series: D. H. R. Barton, D. A. Lewis, and J. F. McGhie, *J. Chem. Soc.*, 2907 (1957).



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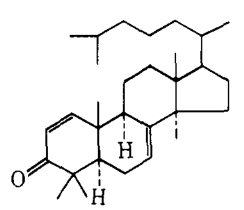
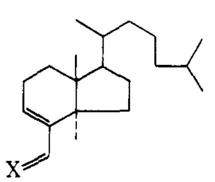


6

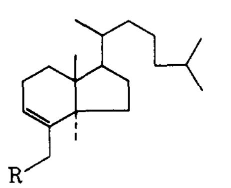
(liquid N_2) receiver as a viscous yellow oil and purified by column chromatography (silica gel, 23% conversion). Spectral properties observed for diene **7** are: $[\alpha]^{20}D$ ($CHCl_3$) +40.6°; ir (neat) 1616, 980, 890, 880 cm^{-1}; λ_{max}^{EtOH} 233 nm (ϵ 8000); nmr (CCl_4) δ 4.77 (dd, J = 2, 11 Hz), 5.13 (dd, J = 2, 17 Hz), 6.03 (dd, J = 11, 17 Hz), 5.50 (m, vinyl protons); mass spectrum *m/e* 288 (70%) M^+, 273 (50%) M − CH_3, 175 (100%) M − side chain.

Diene **7** was treated with 1 equiv of OsO_4 in THF for 2 hr at room temperature and the glycol thus formed (78%; $[\alpha]^{20}D$ ($CHCl_3$) +36.4°; ir (neat) 3340, 1060, 780 cm^{-1}; nmr (CCl_4) δ 3.60 (m, $-CH_2OH$), 4.14 (m, −CHOH), 5.57 (m, vinyl H)) was cleaved (quantitative) with $NaIO_4$ in THF to the unsaturated aldehyde **8**: $[\alpha]^{20}D$ ($CHCl_3$) +30.5°; λ_{max}^{EtOH} 236 nm (ϵ 7000); ir (neat) 1690, 1620, 1225, 1195, 1180 cm^{-1}; nmr (CCl_4) δ 6.42 (t, J = 3.5 Hz, vinyl H), 9.30 (s, aldehyde H). $NaBH_4$ reduction (quantitative) of the aldehyde provided allylic alcohol **9** ($[\alpha]^{20}D$ ($CHCl_3$) +25°; ir (neat) 3340, 1240, 1025 cm^{-1}; nmr (CCl_4) δ 3.98 (s, $-CH_2OH$), 5.38 (t, J = 4 Hz, vinyl H)) which was converted to bromide **10** (80%, nmr (CCl_4) δ 3.94 (s, $-CH_2Br$), 5.66 (t, J = 4 Hz, vinyl H)) using 1 equiv of CBr_4 and $(C_6H_5)_3P$ in CH_2Cl_2 at room temperature for 4 hr.

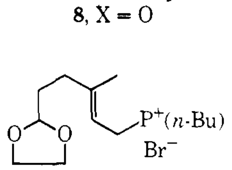
The relatively unstable bromide was allowed to react with the ylide derived from tri-*n*-butylphosphonium salt **11**.[5] The crude coupled phosphonium salt was reduced with Li–$EtNH_2$ at −78° for 3 hr to give acetal **12**: $[\alpha]^{20}D$ ($CHCl_3$) +22.8°; ir (neat) 1135, 1030 cm^{-1}; nmr (CCl_4) δ 1.61 (s, vinyl methyl), 5.09 (m, vinyl H); mass spectrum, *m/e* 430, M^+ (50% overall from **10**).



7, X = CH_2
8, X = O

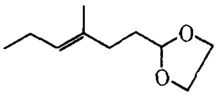


9, R = −OH
10, R = −Br

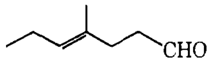


11

12, R =



13, R =



The acetal was hydrolyzed with 3% $HClO_4$ in THF solution at room temperature for 2 days, and aldehyde **13** ($[\alpha]^{20}D$ ($CHCl_3$) +22.6°; ir (neat) 1730 cm^{-1}; nmr (CCl_4) δ 1.61 (s, vinyl methyl), 9.72 (t, J = 3 Hz, aldehyde H)) transformed to epoxide **4** ($[\alpha]^{20}D$ ($CHCl_3$) +20.3°; ir (neat) 1245, 1120 cm^{-1}; nmr (CCl_4) δ 1.61 (s, vinyl methyl), 2.50 (t, J = 6 Hz, epoxide H), 5.06 (m, vinyl protons)), by means of diphenylsulfonium isopropylide (85% from **12**).

Upon treatment with 1 equiv of $SnCl_4$ in benzene at 0°, epoxide **4** gave rise to a variety of products,[6] including in submilligram amount (3–5% conversion) a new tetracycle to which we assign structure **5a** (mp 117–123°; ir ($CHCl_3$)) 3400 cm^{-1}; nmr (CCl_4) δ 3.23 (m, 3α-H), 5.21 (m, 7-H); mass spectrum, 428, M^+) based mainly on the mass spectral and vpc data presented below.

Treatment of **5b** with dry hydrogen chloride in acetic acid at room temperature induced epimerization at C-9, and gave rise to an equilibrium mixture of the (9α-H) Δ^7- and Δ^8-dihydrolanosteryl acetates, as indicated by their vpc behavior on several columns, and a vpc–mass spectral study carried out on the mixture. The mass spectra of the mixture and those of an authentic mixture were superposable. Mercuric acetate in CH_3COOH–EtOH (5 days reflux) converted **5b** to the $\Delta^{7,9(11)}$ compound, identified by vpc and uv comparison with authentic material (λ_{max}^{EtOH} 234, 243, 252 nm (ϵ 15,000, 17,500, 11,500)).[7]

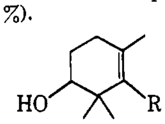
Mass spectral study of **6** revealed a pronounced retro Diels–Alder (RDA) cleavage (*m/e* 288 (70%) RDA, 273 (20%) RDA − CH_3, 175 (57%) RDA − side chain, 137 (100%) ring B cleavage), assignments readily supported by the independent mass spectrum of diene **7** (*m/e* 288 (70%) M^+, 273 (52%) M − CH_3, 175 (100%) M − side chain). Similarly, Δ^1 ketone obtained by C-3 oxidation and C-2 halogenation–dehalogenation (as in preparation of **6**) of dihydrolanosterol isomer **5a** exhibited a mass spectrum where the following ratios apply: *m/e* 288 (25%), 273 (11%), 175 (35%), 137 (100%).[8]

Since Δ^7–Δ^8-dihydrolanosterol and tirucallenol are not detectably produced from epoxide under the conditions described, apparently cyclization through chair–chair conformations is prevented by the severe steric interaction existing between vinyl methyl and *either* angular methyl group in the bicyclic moiety. Chair–boat conformation **4** avoids these restraints and permits formation of the 9,10 cis isomer. Thus, generation of this biochemically crucial stereochemical arrangement can be achieved nonenzymically by means of either conformational constraints or solvent effects,[9] factors which therefore might also assist the biological process. Also, generation of 9β-H Δ^7 isomer **5a**—rather than Δ^8—from **4** indicates that proton loss at C-7 in precursor carbonium ion is kinetically preferred, suggesting that in the enzyme system Δ^8-lanosterol is produced not by spontaneous proton loss but by enzyme-con-

(4) This thermolysis may be an example of a permitted (retro) Diels–Alder reaction ($\pi 4a + \pi 2a$) of a trans-fused hydronaphthalene, as conceived by R. B. Woodward and R. Hoffmann, *Angew. Chem., Int. Ed. Engl.*, **8**, 781 (1969).

(5) E. Axelrod, G. M. Milne, and E. E. van Tamelen, *J. Amer. Chem. Soc.*, **92**, 2139 (1970).

(6) Other identified products are the tricycles i (18%), ii (32%), and iii (21%).



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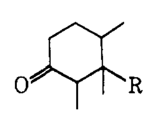
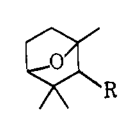


ii



iii

(7) C. Dorée, J. F. McGhie, and F. Kurzer, *J. Chem. Soc.*, 570 (1949).

(8) Satisfactory analytical data have been obtained for all new intermediates.

(9) E. E. van Tamelen and J. P. McCormick, *J. Amer. Chem. Soc.*, **91**, 1847 (1969).

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.

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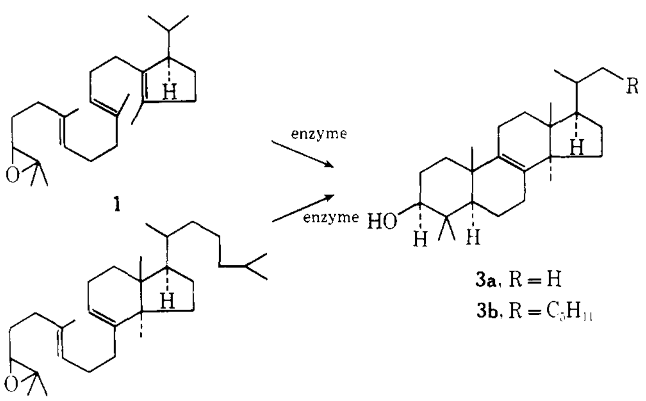
Biochemical Conversion of Partially Cyclized Squalene 2,3-Oxide Types to the Lanosterol System. Views on the Normal Enzymic Cyclization Process

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 × 10^8 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) × 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) × 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).