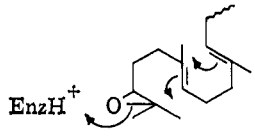
sterol fraction from *anaerobic* incubation of racemic 2,3-oxidosqualene was shown by vpc to consist primarily of material with retention time of lanost-8-en-3β-ol. Lesser amounts of lanosterol (7%) and 4,4-dimethylcholest-8-en-3β-ol (20%) were also indicated.[10,11] The identity of the major product was confirmed by dilution with nonradioactive lanost-8-en-3β-ol and recrystallization to constant specific radioactivity. Furthermore, acetylation of this recrystallized material produced an acetate whose specific radioactivity was not changed by plc on silica gel impregnated with silver nitrate under conditions[12] which readily resolve, for example, a synthetic mixture of 3β-acetoxylanost-8-ene and lanosteryl acetate (3β-acetoxylanosta-8,24-diene). Lastly, aerobic incubation of this radioactive product (as the sterol) afforded a radioactive mixture of cholesterol and cholest-7-en-3β-ol.

Experimental evidence has also been obtained for the metabolic formation of 2,3-oxidosqualene from squalene in rat liver under the conditions of sterol synthesis. Radioactive squalene and nonradioactive racemic 2,3-oxidosqualene (ratio 1:175) were incubated with rat liver homogenate under the standard conditions until *ca.* one-half of the squalene was converted to more polar substances (30 min required). Subsequent isolation of 2,3-oxidosqualene by plc afforded chromatographically homogeneous oxide which was radioactive at a level indicating that 3% of the initially used squalene was recovered as oxide. To confirm the identity of the radioactive product the oxide was subjected to perchloric acid catalyzed hydration under conditions which lead to 2,3-dihydroxysqualene. The chromatographic behavior of the radioactive product so obtained was very different from 2,3-oxidosqualene as expected and *identical* with that of authentic 2,3-dihydroxysqualene.

Thus, it has been demonstrated that 2,3-oxidosqualene is synthesized from squalene in the sterol-forming rat liver system and, further, that this substance is a precursor of sterols which is, in fact, far more efficiently incorporated than is squalene under anaerobic conditions. Therefore it seems likely that sterol synthesis, at least in rats and probably more generally, involves the intermediate 2,3-oxidosqualene (I) which is cyclized by a mechanism such as




We plan to pursue this investigation along a number of lines which are suggested by the above results.

**Acknowledgments.** It is a pleasure to acknowledge numerous helpful discussions with Professors T. T. Tchen and Konrad Bloch and the valuable experimental assistance of Mr. Bruce Ganem. This work was generously supported by the National Science Foundation and the National Institutes of Health.

**E. J. Corey, William E. Russey, Paul R. Ortiz de Montellano**

*Department of Chemistry, Harvard University*
*Cambridge, Massachusetts 02138*

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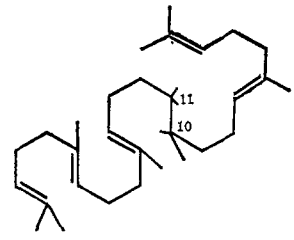
## Metabolic Fate of 10,11-Dihydrosqualene in Sterol-Producing Rat Liver Homogenate

*Sir:*

The generally held view that the biological conversion of squalene to sterols involves a folded conformation of the substrate which is ideal for concerted cyclization to form consecutively the A, B, C, and D rings of the steroid nucleus[1,2] raises the question of whether slight modification of the squalene structure would prevent completely the operation of the cyclizing enzyme or lead to nonsteroidal cyclic transformation products. It appeared of interest to study this point with a substrate such as all-*trans*-10,11-dihydrosqualene[3] (I), which can be folded into a shape approximating, though *not* identical with, the proposed reactive conformation of squalene, but which can give rise at most to only two of the four rings of the steroid nucleus. The results of such an investigation with I are described herein.

All-*trans*-10,11-dihydrosqualene (I) was synthesized by the addition of the Grignard reagent from *trans*-2,3-dihydrofarnesyl bromide[4] to *trans,trans*-farnesal (from *trans,trans*-farnesol by oxidation with $MnO_2$) to give 13-hydroxy-10,11-dihydrosqualene and subsequent successive replacement of the 13-hydroxyl group by bromine ($PBr_3$) and hydrogen ($LiAlH_4$). The synthetic hydrocarbon I was carefully purified *via* the crystalline thiourea complex. All-*trans*-[13-$^3$H]-10,11-dihydrosqualene was synthesized using the same sequence but with lithium aluminum tritide as the reagent in the last step.




I

When [13-$^3$H]10,11-dihydrosqualene was incubated (aerobically) with rat liver homogenate,[5] conversion to

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

(2) For a general review see R. B. Clayton, *Quart Rev.* (London), **19**, 168 (1965).

(3) The nomenclature used here is derived from the numbering of the carbon atoms in the squalene chain 1–24, starting and ending with the terminal methyl groups.

(4) Farnesoic acid was reduced by sodium in amyl alcohol at reflux to give *trans*-2,3-dihydrofarnesoic acid, and further reduced by lithium aluminum hydride to *trans*-2,3-dihydrofarnesol. This in turn afforded the corresponding bromide *via* the *p*-toluenesulfonate using lithium bromide in a displacement reaction.

(5) N. L. R. Bucher and K. McGarrahan, *J. Biol. Chem.*, **222**, 1 (1956).

(9) All experiments were performed at least in duplicate; reproducibility of results was excellent.

(10) It has been reported that under anaerobic conditions with rat liver homogenate lanosterol is reduced efficiently to lanost-8-en-3β-ol: J. Avigan, D. S. Goodman, and D. Steinberg, *J. Biol. Chem.*, **238**, 1283 (1963).

(11) The conversion of lanosterol to cholesterol by rat liver homogenate has been shown to require oxygen by J. A. Olson, Jr., M. Lindberg, and K. Bloch, *ibid.*, **226**, 941 (1957), and cholesterol is not expected to be produced from 2,3-oxidosqualene under anaerobic conditions.

(12) F. C. den Boer, *Z. Anal. Chem.*, **205**, 308 (1964).

nonhydrocarbon products occurred at about three-fourths the rate found for squalene under the same conditions. Chromatographic analysis using the thin layer technique (tlc) on silica gel revealed that there was very little product in the $R_f$ range expected for sterols or monohydroxy $C_{30}$ molecules and that the major products exhibited higher $R_f$ values, as expected for substances considerably less polar than sterols. In fact, the chromatographic behavior of the principal product corresponded closely to that of 2,3-oxidosqualene,[6] which suggested that it might be a terminal epoxidation product of 10,11-dihydrosqualene. This was shown to be the case by synthesis of a mixture of 2,3-oxido- and 22,23-oxido-10,11-dihydrosqualene using the bromohydrin method[6,7] starting with 10,11-dihydrosqualene,[8] and comparison with the biologically produced material. The biological and synthetic samples were identical chromatographically using tlc, with different solvent systems on silica gel and silica gel impregnated with silver nitrate, and vapor phase chromatography. Perchloric acid catalyzed hydration of these samples gave products which behaved identically in tlc and exactly as expected for $C_{30}$ vicinal glycol.[6] Finally, the mass spectra of the biological and synthetic oxides were identical and left no doubt that each sample consisted of approximately equal amounts of the two possible terminal epoxidation products.

A second product, of significantly greater polarity than the terminal oxides of I, was present in the biologically produced mixture from 10,11-dihydrosqualene. This was isolated by preparative layer chromatography and analyzed by mass spectrometry, which allowed identification as 2,3:22,23-dioxido-10,11-dihydrosqualene.[9]

These results, coupled with the finding that 2,3-oxidosqualene is a precursor of sterols in the rat liver system,[6] suggest that the enzyme responsible for the terminal epoxidation of squalene functions almost as well to catalyze epoxidation at either terminal double bond of 10,11-dihydrosqualene. Further, it is evident that the cyclizing enzyme which converts 2,3-oxidosqualene to a steroidal structure rejects both of the terminal oxides from 10,11-dihydrosqualene and allows these to accumulate when 10,11-dihydrosqualene is employed as a substrate in the rat liver system. Thus, some evidence has been provided for the high degree of specificity expected for the steroid-forming enzyme on the basis of the concerted cyclization scheme with a rigidly held, folded[1] substrate.

Lastly, it should be mentioned that 10,11-dihydrosqualene diminishes the rate of conversion of squalene to sterols with rat liver homogenate. However, a comparable effect was observed with squalane and even *n*-eicosane, which indicates that the apparent competitive inhibition is quite nonspecific with regard to structure.

(6) E. J. Corey, W. E. Russey, and P. R. Ortiz de Montellano, *J. Am. Chem. Soc.*, **88**, 4750 (1966).
(7) E. E. van Tamelen and T. J. Curphey, *Tetrahedron Letters*, 121 (1962).
(8) The epoxide obtained from 10,11-dihydrosqualene *via* the monobromohydrin (purified chromatographically) could not be resolved chromatographically. However, it was readily apparent from the mass spectrum that it was a mixture of the two possible *terminal* monooxides, 2,3-oxido- and 22,23-oxido-10,11-dihydrosqualene, in approximately equal amounts.
(9) The ratio of monooxide to dioxide from 10,11-dihydrosqualene under the biological conditions used was *ca.* 5.

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**E. J. Corey, William E. Russey**
*Department of Chemistry, Harvard University*
*Cambridge, Massachusetts 02138*
*Received August 11, 1966*

## Enzymic Conversion of Squalene 2,3-Oxide to Lanosterol and Cholesterol

*Sir:*

The role of the $C_{30}$ triterpenoid hydrocarbon, squalene (I), as an intermediate in the biosynthesis of cholesterol and of the naturally occurring tetracyclic and pentacyclic triterpenes is well supported by experimental evidence.[1]

On a theoretical level, past contributors[2,3] considered the biological formation of the polycyclic triterpenoids from squalene by a mechanism in which cyclization was initiated by attack of $OH^+$ on one of the terminal double bonds of the hydrocarbon chain and proceeded in a concerted fashion without the formation of any stable intermediates. The structure of the product, according to these proposals, would be determined by the conformation imposed upon the squalene chain by the enzyme catalyzing the cyclization. The rearrangements of methyl groups and hydrogen atoms of squalene which were presumed to occur during its cyclization to lanosterol were rationalized on the basis of conformational stereoelectronic considerations. Several features of these proposals have been tested by experiment.

The results of Tchen and Bloch[4] supported the concerted mechanism for this conversion, and convincing evidence for the postulated shifts of methyl groups and hydride ions has also been obtained.[5-7] The precise role of oxygen in the cyclization process has not been investigated, however, although it has been demonstrated by Tchen and Bloch[8] that atmospheric oxygen provides the 3$\beta$-hydroxyl group of lanosterol and that NADPH is required in the reaction. While these observations are consistent with cyclization mechanism A, they do not exclude other possibilities. One of these is mechanism B in which oxygen first adds to the terminal double bond of squalene to yield squalene 2,3-oxide which then undergoes a proton-initiated cyclization.

It recently became possible to test mechanism B when a procedure was developed in one of our laboratories[9]

(1) R. B. Clayton, *Quart. Rev.* (London), **19**, 168 (1965).
(2) (a) L. Ruzicka, *Experientia*, **9**, 357, 362 (1953); (b) A. Eschenmoser, L. Ruzicka, O. Jeger, and D. Arigoni, *Helv. Chem. Acta*, **38**. 1890 (1955).
(3) G. Stork and A. Burgstahler, *J. Am. Chem. Soc.*, 77, 5068 (1955),
(4) T. T. Tchen and K. Bloch, *J. Biol. Chem.*, **226**, 931 (1957).
(5) R. K. Maudgal, T. T. Tchen, and K. Bloch, *J. Am. Chem. Soc.*, 80, 2589 (1958).
(6) J. W. Cornforth, R. H. Cornforth, A. Pelter, M. G. Horning, and G. Popjak, *Tetrahedron*, **5**, 311 (1959).
(7) J. W. Cornforth, R. H. Cornforth, C. Donninger, G. Popjak, Y. Shimizu, S. Ichii, E. Forchielli, and E. Caspi, *J. Am. Chem. Soc.*, **87**, 3224 (1965).
(8) T. T. Tchen and K. Bloch, *J. Biol. Chem.*, **226**, 921 (1957).
(9) E. van Tamelen and T. J. Curphey, *Tetrahedron Letters*, **3**, 121 (1962).