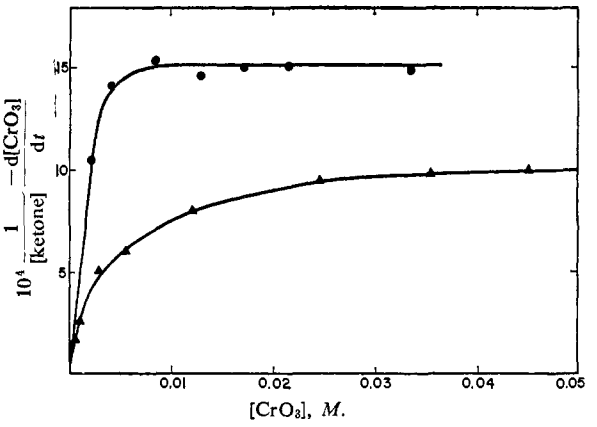


Figure 1. Effect of the initial concentration of chromic acid on the rate of oxidation at 30°. Circles, isobutyrophenone in 0.50 M $HClO_4$ in 99% acetic acid; triangles, 2-chlorocyclohexanone in 1.0 M aqueous $HClO_4$.

and therefore very strongly support the enolization mechanism.

As these results were obtained from two structurally very different ketones in two different solvent media and as no monoketone is known to undergo oxidation which is considerably faster than its enolization,[7] we believe these results support the assumption that enolization generally precedes oxidation in the reaction of chromic acid with a monoketone. In this respect then chromic acid reacts similarly as thalic, mercuric, and permanganate salts[8] and also as manganic pyrophosphate,[9] but differs from cobaltic[10,11] and ceric[11,12] salts and from manganic sulfate[11] as oxidants.

Acknowledgment. Support of this research through No. 1524-A4, a grant of the Petroleum Research Fund of the American Chemical Society, is gratefully acknowledged.

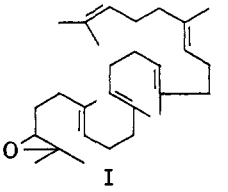
(7) Sr. A. Riehl, Thesis, The Catholic University of America, Washington, D. C., 1965.
(8) J. S. Littler, *J. Chem. Soc.*, 827 (1962).
(9) A. Y. Drummond and W. A. Waters, *ibid.*, 497 (1955).
(10) D. G. Hoare and W. A. Waters, *ibid.*, 971 (1962).
(11) J. S. Littler, *ibid.*, 832 (1962).
(12) S. Venkatakrishnan and M. Santhappa, *Z. Physik. Chem.* (Frankfurt), 16, 73 (1958).
(13) Address correspondence to Department of Chemistry, University of Illinois at Chicago Circle, Chicago, Ill. 60680.

Jan Roček,[13] Sr. Agatha Riehl
Department of Chemistry, The Catholic University of America
Washington, D. C. 20017
Received July 18, 1966

2,3-Oxidosqualene, an Intermediate in the Biological Synthesis of Sterols from Squalene[1]

Sir:

There is now ample evidence for the role of squalene as a precursor of sterols and polycyclic triterpenes and for the relationships between the carbon skeleton of squalene and the structures of many of the polycyclic natural products derived therefrom.[2] However, much remains to be learned about the details of these remarkable cyclization processes. This note is concerned with the question of how cyclization of squalene is initiated and with the connection between this cyclization process and the origin of the characteristic 3-hydroxyl group of sterols and triterpenes. There are three general types of mechanisms which can reasonably account for the cyclization of squalene and the introduction of the 3-hydroxyl substituent: (1) attack at C_3 by a reagent which transfers OH^+ with concurrent initiation of cyclization from C_2;[3] (2) proton transfer to C_3 to initiate cyclization to a 3-deoxycyclosqualene followed by subsequent hydroxylation at the saturated 3-methylene group of the cyclization product;[4] and (3) introduction of oxygen at C_3 to give an intermediate which is capable of cyclization in a separate step. Evidence has been obtained which strongly supports the last of these propositions and indicates specifically



I

that the 2,3-oxidosqualene (I) is an intermediate in the biosynthesis of sterols from squalene.

The biosynthetic experiments were carried out using rat liver homogenates[5] and a number of radioactive substrates, including [^{14}C]squalene[6] and racemic [^{14}C]2,3-oxidosqualene (I).[7]

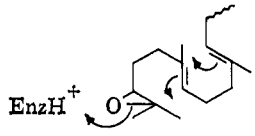
Using rat liver homogenate under standardized aerobic conditions which convert squalene to sterols, racemic [^{14}C]2,3-oxidosqualene gave rise to a radioactive sterol fraction which was isolated by preparative layer chromatography (R_f corresponding to cholesterol) and shown by vapor phase chromatography (vpc) and comparison with authentic samples to consist of cholesterol and lathosterol (cholest-7-en-3β-ol) in a ratio of *ca.* 1:2.[8] Under these aerobic conditions, which gave 62% conversion of [^{14}C]squalene to sterols, 40% of the racemic [^{14}C]2,3-oxidosqualene was transformed into labeled sterols (80% conversion based on a *single* antipode). The conversion of [^{14}C]2,3-oxidosqualene to sterols was essentially unchanged when the standard incubation was carried out *anaerobically* under argon whereas the incorporation of [^{14}C]squalene into sterols dropped to only 13% in the anaerobic system.[9] The

(1) In this note the names used for squalene derivatives have been obtained by assigning the numbers 1–24 to the carbons along the chain from one terminal methyl group to the other. Thus, 2,3-oxidosqualene corresponds to 2,3-oxido-2,6,10,15,19,23-hexamethyl-6,10,14,18,22-tetracosapentaene.
(2) For a recent review, see R. B. Clayton, *Quart. Rev.* (London), 19, 168 (1965).
(3) See L. Ruzicka, A. Eschenmoser, and H. Heusser, *Experientia*, 9, 357 (1953); A. Eschenmoser, L. Ruzicka, O. Jeger, and D. Arigoni, *Helv. Chim. Acta*, 38, 1890 (1955).
(4) Arguments in favor of this possibility have been given recently by D. H. R. Barton and G. P. Moss, *Chem. Commun.*, 261 (1966).
(5) Prepared by the method of N. L. R. Bucher and K. McGarrahan, *J. Biol. Chem.*, 222, 1 (1956).
(6) Prepared by anaerobic incubation of rat liver homogenate[5] with DL-[2-^{14}C]mevalonic acid dibenzylethylenediamine salt and purified chromatographically.
(7) [^{14}C]Squalene[6] was converted to the 2,3-bromohydrin using the method of E. E. van Tamelen and T. J. Curphey, *Tetrahedron Letters*, 121 (1962), which employs hypobromous acid in aqueous glyme. The monobromohydrin was purified by preparative layer chromatography (plc) on silica gel and transformed by treatment with ethanolic base to racemic [^{14}C]2,3-oxidosqualene, homogeneous on thin layer chromatography. An analysis of the oxide by high-resolution mass spectrometry indicated a molecular weight of 426.3860 (calcd for $C_{30}H_{50}O$: 426.3861).
(8) Vpc analyses were conducted on an F & M Model 400 biomedical instrument using a glass column (3 mm i.d.) 4 ft in length packed with 2% Epon 1001 on Diatoport S at 235°.

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

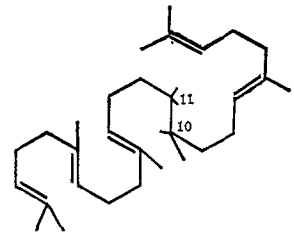
Received August 9, 1966

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).