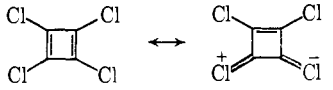
among the six possible directions and consequent observation of averaged atomic coordinates. The possibility of the molecule having different structures in the solid state and in solution is ruled out by nearly identical solution and KBr pellet ir spectra.

The stereospecificity of the additions observed so far and the effectiveness of electron-rich[11] olefins in intercepting the intermediate lead us to postulate that the reactive species is singlet tetrachlorocyclobutadiene. A control experiment showed that cyclohexadiene does not add to IV under the reaction conditions, and we would not expect the pentachlorocyclobutenyl anion to add to either styrene or cyclohexadiene.

This strikingly straightforward synthesis of a substituted cyclobutadiene probably rests heavily on the ability of chlorine both to accept and to donate electron density, with resulting "push–pull"[12] stabilization as indicated.



Further work on the preparation and transformations of V and its adducts is in progress.

(11) J. Sauer and H. Wiest, *Angew. Chem. Intern. Ed. Engl.*, **1**, 269 (1962).

(12) R. Breslow, D. Kivelevich, M. J. Mitchell, W. Fabian, and K. Wendel, *J. Amer. Chem. Soc.*, **87**, 5132 (1965).

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Separation of the Cyclization and Rearrangement Processes of Sterol Biosynthesis. Enzymic Formation of a Protosterol Derivative

Sir:

The biosynthesis of sterols is presumed to involve cyclization of 2,3-oxidosqualene[1,2] to the cation **1** (or its functional equivalent) and subsequent rearrangement by a sequence of 1,2 shifts to lanosterol (**2**).[3,4] The driving force for the over-all rearrangement comes at least in part from the relief of repulsive interactions in **1** which are associated with the B ring (obligatory twist-boat geometry) and its substituents, especially the methyl group attached to C-8. This communication reports the results of a study of the action of 2,3-oxidosqualene–sterol cyclase on the unnatural substrate **3** which by the normal mode of cyclization would produce the cation **4**, a structure lacking the methyl substituents at C-8 and C-14 of **1** and, therefore, less strained and less apt to rearrange to a lanosterol analog. This study demonstrates that the structure (**4**) produced by cyclization of the substrate **3** undergoes proton elimination to form the *unrearranged protosterol derivative* **10**.[5]

(1) (a) E. J. Corey and W. E. Russey, *J. Amer. Chem. Soc.*, **88**, 4751 (1966); (b) E. J. Corey, W. E. Russey, and P. R. Ortiz de Montellano, *ibid.*, **88**, 4750 (1966); (c) W. E. Russey, Ph.D. Thesis, Harvard University, 1966.

(2) (a) E. E. van Tamelen, J. D. Willet, R. B. Clayton, and K. E. Lord, *J. Amer. Chem. Soc.*, **88**, 4752 (1966); (b) J. D. Willet, K. B. Sharpless, K. E. Lord, E. E. van Tamelen, and R. B. Clayton, *J. Biol. Chem.*, **242**, 4182 (1967).

(3) R. B. Woodward and K. E. Bloch, *J. Amer. Chem. Soc.*, **75**, 2023 (1953).

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

(5) The term protosterol is used here to mean the tetracyclic system corresponding to the presently hypothetical precursor **1**. Fusidic acid [W. O. Godtfredsen, W. von Daehne, S. Vangedal, A. Marquet, D. Arigoni, and A. Malera, *Tetrahedron*, **21**, 3505 (1965)], helvolic acid [S. Okuda, S. Iwasaki, M. I. Sair, Y. Machida, A. Inoue, and K. Tsuda, *Tetrahedron Lett.*, 2295 (1967)], and cephalsporin P_1 [T. G. Halsall, E. R. H. Jones, G. Lowe, and C. E. Newall, *Chem. Commum.*, 685 (1966)] represent specific natural products in the protosterol (fusidane) series.

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

(7) E. J. Corey, J. A. Katznellenbogen, and G. H. Posner, *J. Amer. Chem. Soc.*, **89**, 4245 (1967).

(8) J. M. Osbond, *J. Chem. Soc.*, 5270 (1961).

(9) See E. J. Corey, M. F. Semmelhack, and L. S. Hegedus, *J. Amer. Chem. Soc.*, **90**, 2416 (1968), and references therein cited.

(10) The coupling process produced a mixture of three geometrical isomers, differing at the two central (disubstituted) olefinic linkages, *trans,trans* (desired product, **5**), *trans,cis*, and *cis,cis* in a ratio of 90:9:1 [gas chromatographic (gc) analysis using Epon 1001 on Diatoport S (F & M Co.) as stationary phase].

(11) Infrared, nuclear magnetic resonance, and mass spectra of **3** and **5** were fully consistent with the assigned structures.

(12) E. J. Corey, K. Lin, and M. Jautelat, *J. Amer. Chem. Soc.*, **90**, 2724 (1968).

(13) The solution of enzyme was prepared by a modification of the method previously described [P. D. G. Dean, P. R. Ortiz de Montellano, K. Bloch, and E. J. Corey, *J. Biol. Chem.*, **242**, 3014 (1967)] which has been developed in these laboratories by Dr. Shozo Yamamoto; see P. Ortiz de Montellano, Ph.D. Thesis, Harvard University, 1968, p 106.

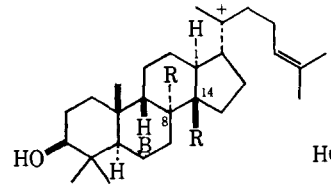
of the labeled product was obtained using a mass spectrometer (LKB Instrument Co.) coupled to a gc apparatus fitted with an OV-1 column[15] which cleanly separated the gc peaks due to cholestanyl acetate and product. The mass spectrum of the acetate showed a molecular ion at *m/e* 440 and major fragments corresponding to loss of 15 (CH_3), 60 (CH_3COOH), and 75 (CH_3 and CH_3COOH) as expected for structure **10** which has been derived for the enzymic product from **3**. In accord with the steroidal formulation **10** is the finding that the formation of the transformation product was completely prevented when 2,3-iminosqualene, a potent inhibitor of the enzyme 2,3-oxidosqualene–sterol cyclase,[16] was added to solutions of the enzyme and the substrate **9**.

It has also been demonstrated that in the conversion of the labeled substrates **8** and **9** to the enzymic cyclization product no tritium is lost. Specifically, a mixture of ^{14}C-labeled **3** and **8** having a ratio $^3H/^{14}C$ of 1.65 yielded enzymic product in which this ratio was 1.62, and a mixture of ^{14}C-labeled **3** and **9** having a ratio $^3H/^{14}C$ of 1.48 afforded enzymic product of $^3H/^{14}C$ ratio of 1.47. This result allows the exclusion of a structure analogous to lanosterol and a number of other partially rearranged structures with a double bond in the steroid nucleus, *e.g.*, Δ13,14 or Δ8,14 derivatives.

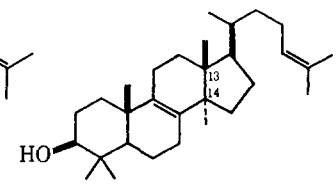
Further information regarding the cyclase transformation product from **3** was obtained by chemical degradation. Hydroxylation of the tritium-labeled acetate of the cyclase product with a very large excess of osmium tetroxide in ether–pyridine solution for 40 hr at 25° followed by glycol cleavage with excess sodium periodate in ethanol–water for 18 hr at 25° afforded after tlc on silica gel with 10% ethyl acetate in benzene a labeled product (91% yield) of R_f 0.32 whose chromatographic behavior was indicative of the presence of a single carbonyl function in addition to the acetoxy group. Especially revealing was the fact that this product was chromatographically indistinguishable from 3β-acetoxy-5-pregnen-20-one. Treatment of the oxidation product under drastic conditions with alkaline silver oxide did not result in the generation of acidic material, arguing against the presence of an aldehyde function. Reaction with alkaline sodium hypoiodite, however, did afford an acidic product (71% yield), as would be expected for a methyl ketone of structure **11**. The acid generated by hypoiodite cleavage, for which structure **12** follows, was converted to a neutral product **13** by treatment with ethereal diazomethane. This ester, R_f 0.38 using silica gel tlc with 10% ethyl acetate in benzene as solvent, was converted to an acetate with acetic anhydride–pyridine which was shown to be totally inert to osmium tetroxide. The mass spectrum[15] of the hydroxy methyl ester clearly indicated structure **13**, the molecular ion occurring at *m/e* 348 with important additional peaks representing loss of the fragments H_2O, CH_3OOC, CH_3, and $C_3H_4COOCH_3$. Although these data do not allow independent confirmation of the stereochemistry, they do provide an unambiguous argument for the gross protosterol structure **10** for the enzymic cyclization product from **3**. Assuming that 2,3-oxidosqualene–sterol cyclase operates without stereochemical variability, the stereochemistry follows as shown in structure **10**.

During the final stages of this work, a publication appeared which described the action of rat liver cyclase on the 2,3-oxidonorsqualene having hydrogen in place of methyl at C-15 to form the norlanosterol lacking methyl at C-13, *i.e.*, the normal cyclization–rearrangement sequence.[17]

There are a number of interesting aspects of the biosynthesis of **10** from **3** which can be posed as questions for future research. (1) Is the further rearrangement to the lanosterol series prevented mainly because of the altered internal energetics of the substrate **3** or because of an effect on the enzyme–substrate interaction? (2) Is the rearrangement process the result of conformational (and other) changes in the cyclizing enzyme or of catalysis by a different enzyme? (3) Would the 2,3-oxidonorsqualene having H in place of CH_3 at C-10 be converted by 2,3-oxidosqualene–sterol cyclase to a norlanosterol or a norprotosterol? Further study relevant to these points is planned.



1, R = CH_3
4, R = H



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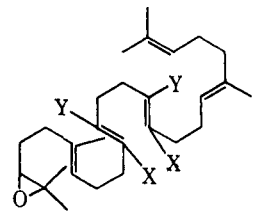
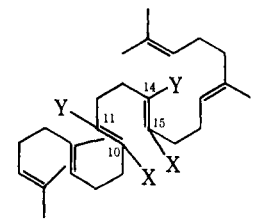
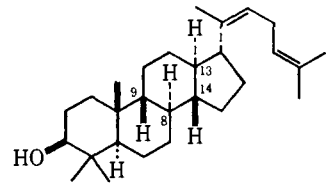


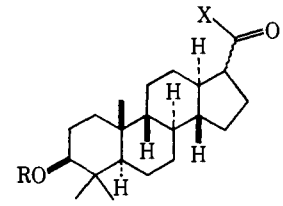
3, X = Y = H
8, X = ^3H; Y = H
9, X = H; Y = ^3H



5, X = Y = H
6, X = ^3H; Y = H
7, X = H; Y = ^3H



10



11, X = CH_3; R = CH_3CO
12, X = OH; R = H
13, X = OCH_3; R = H

Acknowledgment. This work was supported financially by the National Institutes of Health and the National Science Foundation.

(14) Using the same gc conditions, t_r values (min) were 24.0 for cholesteryl acetate, 33.0 for 24,25-dihydrolanosteryl acetate, and 36 for lanosteryl acetate.

(15) We are indebted to Dr. James Orr, Massachusetts General Hospital, for the mass spectral data and to Dr. Albert Schonberg of Merck Sharp and Dohme Research Laboratories for a preliminary measurement.

(16) E. J. Corey, P. R. Ortiz de Montellano, K. Lin, and P. D. G. Dean, *J. Amer. Chem. Soc.*, 89, 2797 (1967).

(17) E. E. van Tamelen, R. P. Hanzlick, K. B. Sharpless, R. B. Clayton, W. J. Richter, and A. L. Burlingame, *ibid.*, 90, 3284 (1968). The substrate is misleadingly described in this paper as "15-norsqualene 2,3-oxide."

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