Completing the Puzzle of Steroid Synthesis

A mystery in the biological synthesis of steroids is solved, providing a new approach to chemical synthesis of the compounds

The last piece in the complicated jigsaw puzzle that is the biological production of steroids has been fit into place by Eugene E. van Tamelen and his colleagues at Stanford University. "We now have," he says, "a reasonably reliable, almost cinematographic picture of the whole process involved in the cyclization of squalene oxide to lanosterol." The newly published results,* says Ian Scott of Texas A & M University, represent "a significant advance in understanding the cyclization process."

Lanosterol is the core steroid from which all others are derived by biological modification—"the Adam of steroids," van Tamelen calls it. It is a complex tetracyclic molecule containing three six-membered rings (labeled A, B, and C) and a five-membered ring (labeled D). The rough outline of the biological pathway for synthesis of lanosterol (and, ultimately, cholesterol) from the two-carbon fragments of acetyl CoA was described in the 1940's and 1950's by Konrad Bloch, John Cornforth, and Feodor Lynen.

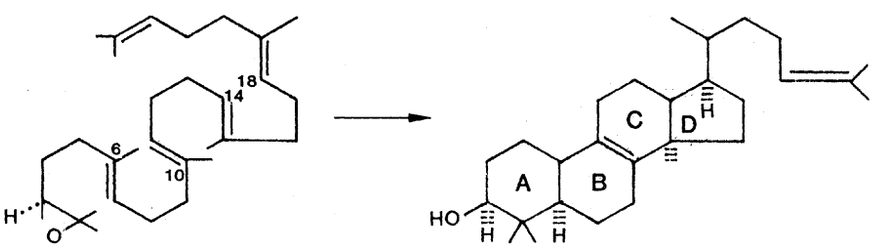
The immediate precursor of lanosterol, as shown 20 years ago by E. J. Corey of Harvard University and van Tamelen, is 2,3-oxidosqualene, a 30-carbon, linear molecule containing five double bonds and an epoxide moiety. This compound is converted directly to lanosterol in a concerted reaction by an enzyme, 2,3-oxidosqualene lanosterol cyclase, that has not yet been fully purified. This cyclization, Bloch says, "has been called the most complex enzyme-catalyzed reaction there is." Over the last 20 years, several investigators, but most especially van Tamelen, have been able to explain all but one step in the reaction.

The reaction proceeds through a series of carbonium ions that form each ring sequentially. The double bond between carbons-6 and -7 attacks the epoxide to form the first ring and generate a carbonium ion at carbon-6. This ion is attacked by the double bond between carbons-10 and -11 to form the second ring and generate a new carbonium ion at carbon-10. This sequence continues until all four rings are formed. After formation of the tetracyclic structure, a number of methyl and hydrogen migrations and a hydrogen elimination occur to form lanosterol.

But by the rules of chemical reactions, van Tamelen says, ring C should be five-membered rather than six. In particular, formation of a five-membered ring would produce a very stable tertiary carbonium ion, whereas formation of a six-membered ring produces a less stable secondary carbonium ion. Attempts at a practical chemical synthesis of lanosterol and its derivatives, in fact, have been frustrated by this tendency toward formation of a five-membered ring. The enzyme thus appears able to bend the chemical rules, but how it does so has been a mystery. van Tamelen's solution to the problem involved synthesis of a squalene variant that lacks a crucial double bond (between carbons-18 and -19) so that cyclization cannot proceed beyond the formation of ring C. The variant also lacks a methyl moiety at carbon-15 so that a secondary carbonium ion is produced regardless of whether a five-membered or six-membered ring is produced.

When this variant, 15′-nor-18,19-dihydrosqualene 2,3-oxide, is acted upon by the enzyme, a tricyclic compound is formed, and each of the rings is six-membered. "What is really important," says van Tamelen, is that a proton is transferred from carbon-18 to carbon-14 during the reaction. Since a spontaneous proton transfer between these two widely separated carbons "is improbable, the two carbons must be held in close proximity by the enzyme; such constraints operating on the natural substrate require that [the carbon in the 14 position and the double bond at the carbon in the 18 position] be similarly juxtaposed, thereby ensuring bond formation between these centers, *contrary to Markovnikov behavior*, as observed in the *nonenzymic* cyclization of squalene oxide." That is, by bringing the two centers close together, the enzyme forces the substrate into a conformation which permits a reaction that would not otherwise occur.

Bloch terms van Tamelen's results "exceedingly interesting and elegant," but he cautions that its "relationship to the biochemical event is necessarily inferential. . . . Ultimately, the answer will have to be obtained with the use of the normal substrate and intermediates"—a prospect that seems remote because the normal reaction cannot be stopped at any intermediate stage.



2,3-Oxidosqualene — Lanosterol

Beyond the "elegance" associated with understanding the reaction mechanism, van Tamelen's results have practical applications. He and his colleagues observed that the 15′-nor-compound cyclizes to a six-membered ring under nonenzymic conditions. This observation and the new understanding of the reaction mechanism "led us to try some chemistry we would not have attempted otherwise." van Tamelen has recently been able to produce lanosterol derivatives from the appropriately substituted oxidosqualenes in one-step reactions with yields of 50 percent or more. These results have not yet been published, but van Tamelen argues that "it will not be possible to devise a simpler method" to synthesize steroids. It is still too soon to tell whether the new synthesis will make it possible to produce commercial steroids more cheaply than current methods. At the very least, however, the new approach should make possible the synthesis of many steroids that are not available.—**THOMAS H. MAUGH II**

*E. E. van Tamelen, E. J. Leopold, S. A. Marson, H. R. Waespe, *J. Am. Chem. Soc.* **104**, 6479 (1982); E. E. van Tamelen, *ibid.*, p. 6480.

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