The enzymic cyclization of 6'-norsqualene-2,3-oxide

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SUMMARY

The present study is a part of an effort to determine the mechanism of action of squalene oxide-lanosterol cyclase. A brief introduction to the biosynthesis of lanosterol is given, together with a survey on the *in vitro* enzymic cyclizations of modified squalene-2,3-oxides. From this introduction the objective of this study becomes clear, this being the determination of the role of the 6'-methyl group in the enzymic cyclization of squalene-2,3-oxide. The approach to this problem is the replacement of the 6'-methyl group by hydrogen and feeding this modified squalene-2,3-oxide to a solubilized and partially purified squalene oxide-lanosterol cyclase from rat liver. The synthesis of radiolabeled racemic 6'-norsqualene-2,3-oxide* is described.

The results from the incubations (*in vitro*) are devided into two categories: (1) small scale incubations with 5 ml of the cyclase solution, (2) large scale incubations with about 100 ml of the cyclase solution. This division was necessitated by the occurrance of two enzymic products. Spectral measurements of the enzymic products, and some chemical transformations from the steroid field together with pertinent spectral data of the derived products, provided evidence for the structure of each of the enzymic products. The product from the large scale incubation proved to be 19-nor-5α-lanosterol, while the product from the small scale incubations was the C10 epimer, *i.e.* 19-nor-5α,10α-lanosterol. A mechanism leading to the product with the unusual A:B-*cis* fused (5α,10α) ring system is proposed.

The conversion efficiency of the substrate to product is shown also to be related to the severity of the non-bonding interaction relieved by removal of a specific methyl group in the unrearranged "C20 carbonium ion".

The occurrance of 19-nor-5α,10α-lanosterol sheds light on some of the characteristics of the cyclizing enzyme. It is concluded that (i) the participation of the enzyme in the termination step cannot be denied, (ii) the removal of the 6'-methyl group might be detrimental to the *in vivo* biosynthesis of lanosterol in rat liver.

* 21-^{3}H-22,23-epoxy-2,6,10,15,23-pentamethyl-2,6,10,14,18-tetracosapentaene