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STEROID BIOSYNTHESIS. RELATIVE EFFICIENCIES OF ENZYMIC TRANSFORMATION OF TERMINALLY MODIFIED SQUALENE 2,3-OXIDE ANALOGS INTO LANOSTEROL ANALOGS BY 2,3-OXIDOSQUALENE CYCLASE

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The incubation of terminally modified squalene 2,3-oxide analogs with the enzyme preparation of hog liver yielded the corresponding lanosterol analogs, respectively, at different conversion rates. The interaction of 2,3-oxidosqualene cyclase with the substrates has thus been found to be influenced by the bulk of the terminal moiety.

Squalene 2,3-oxide (I), but not squalene, has been shown to be the only substrate which suffers the enzymic cyclization to lanosterol by 2,3-oxidosqualene cyclase prepared from rat liver.[1,2] The structural modification in the proximity of the epoxy group of the 2,3-oxide (I) has been revealed to affect the initiation of the enzymic cyclization.[3,4] Enzymic conversions of some terminally modified squalene 2,3-oxide analogs into the corresponding lanosterol analogs[5-8] have shown that the substrate specificity of the enzyme is low. However, a quantitative comparison of the relative efficiency of transformation of squalene 2,3-oxide and its terminally modified analogs has not been done yet. The comparison may be expected to afford some informations on the interaction between the substrate and the enzyme in the enzymic cyclization of the 2,3-oxide and its analogs. We now have incubated squalene 2,3-oxide (I), squalene 2,3:22,23-dioxide (II), 2-hydroxysqualene 22,23-oxide (III), and 3-hydroxy-1,1',2-trisnorsqualene 22,23-oxide (IV) under the same conditions with the cyclase system prepared from hog liver.

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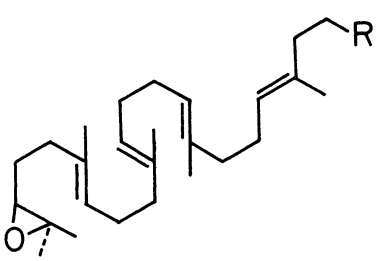
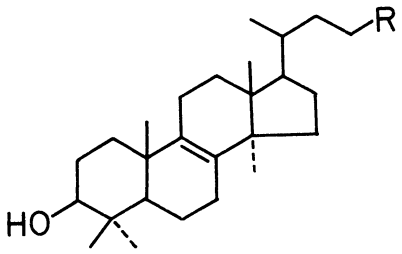
The labeled substrates (I)∿(IV)[9] were synthesized starting from [^{14}C]squalene and their spectral data were in accord with the assigned structure. The fresh hog liver (100 g) was minced with 0.1 M phosphate buffer (100 ml, pH 7.4) and subsequently homogenized in a mortar at 0°C. The resulting homogenate was centrifuged for 30 min at 14,000 × g and 0°C and the supernatant was used for incubation. The radioactive substrate (1.0 mg) suspended in the buffer solution (3 ml) containing Tween 80 (6 mg) was incubated anaerobically at 37°C for 2 hr with the enzyme preparation (3 ml) or with the boiled enzyme system as a control. An incubated mixture was extracted with a mixture of ether and *n*-hexane, after saponification with methanolic potassium hydroxide.



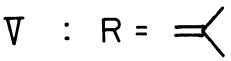


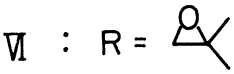
I : R = 

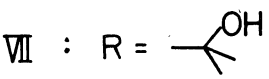
II : R = 

III : R = 

IV : R = CH_2OH

V : R = 

VI : R = 

VII : R = 

VIII : R = CH_2OH

The radio thin-layer chromatographic analyses,[10] using a silica gel plate (Kieselgel G, 0.25 mm thick) and a mixture of ethyl acetate and *n*-hexane as a solvent, revealed the mixture to be composed of an unchanged oxide and a sterol in all cases of the substrates (I)∿(IV). The conversion rate of the substrates to the corresponding sterols was determined from their radioactivities and the results are shown in Table 1. In each case the boiled enzyme control gave less than 2% material in the sterol and other products regions on the radio thin-layer chromatogram. This indicated that the sterols (V)∿(VIII) were produced by the action of the enzyme. The identity of the sterols (V)∿(VIII) to lanosterol, 24,25-oxidolanosterol,[11] 25-hydroxylanosterol,[12] and 24-hydroxy-25,26,27-trisnorlanosterol,[13] respectively, was established by direct

Table 1. Conversion Rate of Some Squalene 2,3-Oxide Analogs to Sterols by 2,3-Oxidosqualene Cyclase.

Substrates	Products Sterols	Radioactivities (%)*	Conversion rates of oxide to sterol (%)**
Squalene 2,3-oxide (I)	(V)	33.1	66.2
Squalene 2,3:22,23-dioxide (II)	(VI)	13.1	26.2
2-Hydroxysqualene 22,23-oxide (III)	(VII)	20.6	41.2
3-Hydroxy-1,1',2-trisnor-squalene 22,23-oxide (IV)	(VIII)	42.0	84.0

* Percentage for counts of the substrate used.

** Calculated as the only (3*S*)-enantiomer of a racemic mixture of the substrate participates in its cyclization to the sterol.[14)]

comparison (mixed m.p., co-TLC, IR, and NMR) with the respective authentic samples.

The conversion rate of the substrates to the corresponding sterols has now been proved to differ from one another; the cyclization of IV is slightly higher than that of 2,3-oxidosqualene (I), whereas the cyclization of II and III is lower than that of I. These results indicate that the interaction between the enzyme and the substrate may be influenced to some extent by the structure of the terminal moiety at the end opposite the epoxy group of the 2,3-oxide (I). It is highly probable that the bulkier terminal moiety the more prevent access of the substrate to the enzyme and this consequently makes the conversion rate progressively reduced.

References and Footnotes

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10) Thin-layer plate radiochromatograms were taken on an Aloka (Tokyo) JTC-203 instrument at a slit width of 1.0 mm.

11) Prepared from lanosterol via its bromohydrin.

12) Prepared from VI on reduction with lithium alminum hydride.

13) Synthesized from the acetate of VI according to such a sequence as the hydrolysis of the oxide to the corresponding 24,25-diol, the sodium periodate oxidation of the diol to a C_{27}-aldehyde, and then the reduction of the aldehyde with lithium aluminum hydride.

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