possible to utilize organoboranes for alkylations of a wide variety of α-halo-substituted carbanions. Moreover, the mechanism also suggests that such alkylations should proceed with retention of the stereochemistry at the migrating center, opening up major new synthetic opportunities. This question is under examination.

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The Role of Substrate Structure in the Initiation of Enzymic Cyclization of Squalene 2,3-Oxide. Studies with 2,3-*cis*-1′-Norsqualene 2,3-Oxide and 2,3-*trans*-1′-Norsqualene 2,3-Oxide

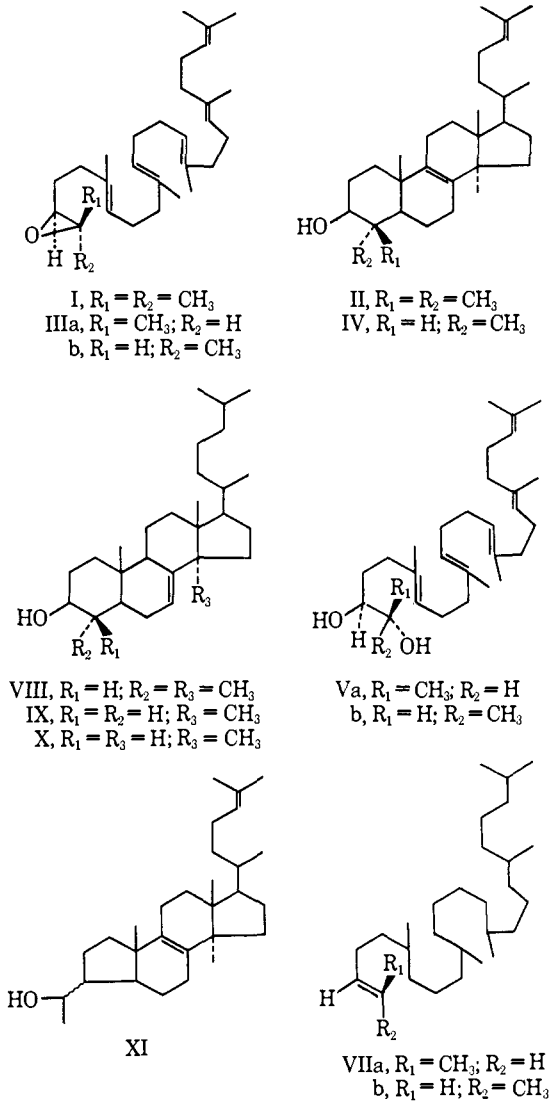
Sir:

Squalene 2,3-oxide (I) is cyclized to lanosterol (II) by a microsomal enzyme system of mammalian liver,[1–4] and squalene 2,3-oxide analogs, modified in the terminal[5,6] and more central[7] portions of the molecule, are also cyclized by this enzyme system. Structural modifications of the squalene 2,3-oxide molecule in the proximity of the oxide ring should exert strong electronic and steric effects upon the initiation of enzymic cyclization. Exploration of these effects may elucidate features of enzyme–substrate interaction that govern this initial phase of cyclization. We have therefore subjected 2,3-*cis*- and 2,3-*trans*-1′-norsqualene 2,3-oxides (IIIa,b) to the action of the cyclase system and have found that only the 2,3-*trans*-oxide IIIb yields a 4-desmethyllanosterol analog (4α,14α-dimethyl-$\Delta^{8,24}$-cholestadien-3β-ol, IV).

2,3-*cis*- and 2,3-*trans*-1′-norsqualene 2,3-oxides (IIIa and IIIb) were prepared from [4-^{3}H]1,1′,2-trisnorsqualene-3-carboxaldehyde[8] by reaction with diethylsulfonium ethylide.[9] The more mobile *trans* isomer was separated from the *cis* by repeated continuous tlc[10] on silica gel with 3% EtOAc–hexane for 2.5 hr. IIIa and IIIb, with $HClO_4$–H_2O, gave the corresponding glycols Va and Vb which afforded the corresponding pure acetonides VIa and VIb. These, unlike the oxides, were stable on glpc[11] on columns of Carbowax, with R_c 1.71 (VIa) and 2.03 (VIb).

The *cis* and *trans* structures of IIIa and IIIb were established by hydrogenation to the corresponding perhydro oxides and conversion to *cis*- and *trans*-alkenes VIIa and VIIb, respectively.[12] These separated on glpc (SE-30) by a factor, *cis*:*trans* = 1.06. The *trans*-alkene VIIb showed a characteristic strong band at 965 cm^{-1} which was absent in VIIa.

IIIa (3.2×10^4 dpm/μg) and IIIb (2.92×10^4 dpm/μg) were incubated with a clarified 100,000*g* supernatant preparation of deoxycholate-treated microsomes of rat liver[13] in 0.08 *M* phosphate buffer, pH 7.4. The substrate (60 μg) was incubated anaerobically at 37° for 1 hr with enzyme solution (3 ml) from 1 g of liver or with boiled enzyme as a control. Products isolated by standard methods[3] and separated by tlc with EtOAc–hexane (1:3) gave materials moving as: (a) unchanged oxide (R_f 0.60–0.67), (b) "sterols" (R_f 0.37–0.45), and (c) "glycol" (R_f 0.11–0.17). Recoveries of radioactivity were *cis*-oxide, IIIa: 80% unchanged, 1.8% "sterol," 15% "glycol," other regions <0.3%; *trans*-oxide, IIIb: 90% unchanged, 3.5% "sterol," 2.2% "glycol," other regions <0.3%. In each case boiled enzyme con-



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trols gave 2.0% material in the "sterol" region with the remainder recovered as unchanged oxides.

The glycols formed enzymically from IIIa and IIIb gave pure acetonides indistinguishable on tlc and glpc from the acetonides VIa and VIb, respectively.

The materials obtained from IIIa and IIIb that moved in the sterol region on tlc were analyzed by glpc on SE-30 with collection of fractions corresponding to nonenzymic (R_c 2.1) and enzymic (sterol) products (R_c 2.1–4.1; R_c for lanosterol, 3.04). Only the *trans*-oxide IIIb yielded a product, IV, in the latter region (R_c 2.54). Essentially all recovered radioactivity in the case of IIIa, as in the boiled enzyme controls, was in the nonsteroidal region (R_c 2.1).

The structure assignment of IV rests on the following observations. The biosynthetic material (as the methyl ether) has R_c 4.52 on DEGS, in close agreement with a calculated value based on retention times of a structurally related series of compounds.[14] On hydrogenation followed by equilibration with dry HCl in $CHCl_3$ IV yielded two products identical in retention times and in molar proportions with those similarly obtained from authentic 4α,14α-dimethyl-Δ⁷-cholesten-3β-ol (VIII)[15] (*i.e.*, 3:1 Δ⁷–Δ⁸, separated on DEGS as the methyl ethers, with R_c 4.07 and 3.12, respectively). Labeled material corresponding to the Δ⁷ derivative VIII was collected from glpc on SE-30 with R_c 2.45–2.95 and cocrystallized with a synthetic sample of VIII as the free sterols and the acetates with no loss of specific activity (sterol, 289–297 and acetate, 238–246 dpm/mg).

The 4α-methyl assignment to IV, rather than 4β-, is to be expected if the stereochemical fate of the methyl group of IIIb is the same as that of the corresponding methyl group in squalene 2,3-oxide. It is supported by the identical behavior on glpc on SE-30 of authentic VIII and the hydrogenated and isomerized product from IV, since we have confirmed[17] the separation reported[18] for 4α- and 4β-methyl sterols on SE-30 (though not on DEGS).

We have considered the possible formation of an alternative product such as the A-nor steroid XI, which should be intermediate in polarity between the sterols and glycols (R_f 0.17–0.37 on tlc). In the ketone derived from XI the labeled hydrogens are β to the keto group and thus, in contrast to those in the ketone corresponding to IV, should be retained under enolizing conditions. Oxidation of material of R_f 0.17–0.37 followed by acid-catalyzed exchange and partition of the products between water and hexane rendered 97–98% of the label water soluble. Moreover no evidence of the formation of products of type XI was revealed by glpc analysis.

These results demonstrate the enzymic cyclization of the *trans*-oxide IIIb, in 1–1.5% yield, to 4α,14α-dimethyl-Δ⁸,²⁴-cholestadienol (IV). The only other identified enzyme product from IIIb was the glycol Vb. The hypothetical alternative cyclization product, the A-nor steroid XI, if formed, can only be present in much smaller amounts. The finding that, in contrast to the *trans*-oxide, the *cis*-oxide IIIa was not measurably cyclized prompts a variety of speculations concerning hitherto unrecognized steric and electronic determinants of the cyclization process, but these can only be fully elucidated by further experiments. Enzymic conversion of IIIb to IV is only 6% of the conversion of squalene 2,3-oxide to lanosterol under identical conditions. Thus, the *gem*-dimethyl structure of the natural substrate is of prime importance in the initiation of enzymic cyclization. The markedly different yields of glycols from IIIa and IIIb, though possibly of significance in relation to cyclase activity, require further study, since preliminary results suggest the presence of a separate oxide hydrolase system.

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A Double Octant Rule for Planar Transition Metal Ion Complexes

In Table I are shown the results of circular dichroism measurements for divalent cupric and nickel ion complexes of tripeptides composed of glycyl and L-alanyl residues. The solutions measured were sufficiently basic that both amide hydrogens had ionized in all tripeptide complexes[1] with the result that amide nitrogens rather than amide oxygens are bound to the metal ions. Numerical values in Table I are the differences in molar absorptivities between left and right circularly polarized light at about 560 mμ for the cupric ion complexes[2] and at about 480 mμ for the yellow square-planar divalent nickel ion complexes. A set of measurements similar to those reported in Table I with leucine substituted for alanine shows near additivity with cupric ion but not with nickel ion.

Table I. Differential Molar Absorptivities of Divalent Metal Ion Complexes of Tripeptides Composed of L-Amino Acid Residues

Tripeptide	Cu[a]	Ni
Gly-Gly-Ala	−0.48	−0.85
Gly-Ala-Gly	−0.75	−1.12
Ala-Gly-Gly	−0.19	−0.11
Sum	−1.42	−2.08
Ala-Ala-Ala	−1.03	−2.10

[a] From ref 2.

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