The Role of Substrate Structure in the Initiation of Enzymic Cyclization of Squalene 2,3-Oxide. Stereochemistry of Homosterol Formation from 1-Methylsqualene 2,3-Oxide

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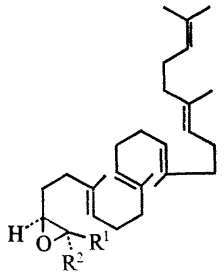
Summary Squalene 2,3-oxide lanosterol cyclase selectively converts the 1′-*cis*-methyl isomer as a component in a mixture of *cis*- and *trans*-1-methylsqualene 2,3-oxides into 4α-ethyl-4β,14α-dimethylcholesta-8,24-dien-3β-ol.

WE have previously described[1] the enzymic cyclization of the 1-*trans*-1′-norsqualene 2,3-oxide analogue (I) into the 4α-methyl-sterol (III) and the lack of detectable cyclization of the corresponding 1-*cis*-derivative, (II). It was noted that, under the same conditions, (I) gave (III) in only 6% of the yield of lanosterol (V) from the natural substrate, squalene 2,3-oxide (IV).

To gain further insight into the factors that determine the different results obtained with substrates (I), (II), and (IV), we have studied the enzymic cyclization of a mixture of the isomers (VI) and (VII), in which a 1-methyl substituent, *cis* or *trans* to the chain, respectively, is replaced by an ethyl group. We report here that cyclization of the *cis*-methyl oxide (VII) proceeds with approximately 45% of the efficiency of squalene 2,3-oxide (IV), but that the *trans*-methyl analogue (VI) is not detectably cyclized.

Tentative evidence for the enzymic formation of a sterol-like product from the mixture of (VI) and (VII) has been reported[2] but without identification either of the product or the metabolically active oxide.

Mixed tritium-labelled 1-methylsqualene 2,3-oxides (VI) and (VII), specific activity 19,000 d.p.m. per μg., were prepared by the reaction of diphenylsulphonium-2-butylide with [4-^{3}H]-1,1′,2-trisnorsqualene-3-aldehyde.[2] The 100 Mc./sec. n.m.r. spectrum of the epoxide in $CDCl_3$ had peaks at 1·220 and 1·255 p.p.m. relative to Me_4Si in the ratio 1 : 2, assigned respectively to the epoxide terminal

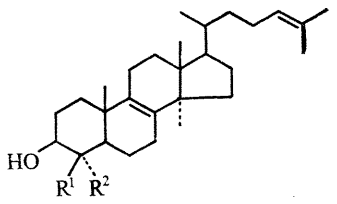


	R^1	R^2
(I)	H	Me
(II)	Me	H
(IV)	Me	Me
(VI)	Et	Me
(VII)	Me	Et
(IX)	H	Et
(X)	Et	H

methyls *cis* (VII) and *trans* (VI) to the hydrocarbon chain by analogy with the n.m.r. spectrum of juvenile hormone.[3]

The mixed epoxides (VI) and (VII) (4·45 mg.) were incubated for 30 min. at 37° with 100 ml. of lanosterol cyclase prepared from microsomes equivalent to 112 g. rat

liver.[1] The nonsaponifiable fraction, extracted with hexane, was separated by t.l.c. on silica gel with ethyl acetate–hexane, 1 : 3. Of the radioactivity recovered from the silica gel, 91·8% corresponded to unchanged epoxide, R_F 0·63, and 8·2% to as terol product, R_F 0·37 (lanosterol R_F 0·38). From control incubations with boiled enzyme, 99·3% of the recovered radioactivity chromatographed as the unchanged oxide.



	R^1	R^2
(III)	H	Me
(V)	Me	Me
(VIII)	Me	Et

The recovered sterol, as the trimethylsilyl ether, when subjected to g.l.c. on DEGS, gave a retention time relative to cholestane (R_c) 3·88 (R_c lanosterol trimethylsilyl ether 3·7). After further purification of the sterol as the acetate by t.l.c., 100 and 60 Mc./sec. n.m.r. spectra of the sterol and a 100 Mc./sec. spectrum of its acetate were obtained. The spectra permit the structural assignment 4α-ethyl-4β,14α-dimethylcholesta-8,24-dien-3β-ol (VIII) to the sterol. Comparison with the spectrum for lanosterol[4] shows that the 4α-methyl peak at 0·97 p.p.m. relative to Me_4Si is missing, while the 4β-methyl peak at 0·81 p.p.m. is still present. In addition, a triplet centred at 0·84 p.p.m. (J 7 c./sec.), overlapping with other peaks, is assigned to the methyl of the 4α-ethyl group. Furthermore, the 100 Mc./sec. n.m.r. spectrum of recovered epoxide shows that the ratio of epoxide with *cis*-terminal methyl to epoxide with *trans*-terminal methyl has changed from the initial value of 1 : 2 to approximately 1 : 3.

These results are consistent with the selective cyclization of the (±)-*cis*-methyl isomer (VII) to the 4β-methylhomosterol (VIII) in approximately 21% yield. Incubations of (±)-squalene 2,3-oxide (IV) with the same enzyme preparation yielded lanosterol in approximately 47% yield, indicating a cyclization efficiency of (VII) about 45% of that of (IV).

The homosterol acetate was hydrogenated over PtO_2 in ethyl acetate solution. A mass spectrum of this hydrogenation product from a separate incubation showed a molecular ion at *m/e* 484 corresponding to a 24-dihydro-derivative of (VIII). Peaks also appeared at 469 (loss of CH_3), 424 (loss of CH_3CO_2H) and 409 (loss of CH_3 and CH_3CO_2H), although the spectrum was somewhat obscured by the presence of an excess of dihydrolanosterol acetate (molecular ion at *m/e* 470) from this particular incubation.

The dihydro-sterol acetate was treated with HCl gas under conditions which equilibrate Δ^8- and Δ^7-isomers of lanosterol.[5] After hydrolysis, the product was converted into its trimethylsilyl ether and subjected to g.l.c. on DEGS. The radioactivity of the sample recovered from the effluent was distributed approximately equally between two peaks with R_c 2·78 (Δ^8-isomer) and 3·33 (Δ^7-isomer) respectively. An essentially identical separation factor (1·31) was obtained under the same chromatographic conditions for the trimethylsilyl ethers of dihydrolanosterol and its Δ^7-isomer.

Another study in these laboratories has shown[6] that enzymic cyclization of squalene 2,3-oxide to lanosterol occurs with no net rotation about the C-2–C-3 bond. Hence, the finding that in the presence of squalene 2,3-oxide lanosterol cyclase, the mixture of geometrical isomers (VI) and (VII) yields the 4β-ethyl-sterol (VIII) indicates that the *cis*-methyl compound (VII), but not its *trans*-methyl isomer (VI), undergoes enzymic cyclization. The n.m.r. data for the unreacted oxides remaining after the enzymic cyclization confirm this conclusion.

These results and those of our previous studies of the norsqualene oxides (I) and (II) reveal a subtle interaction of electronic and steric forces that control the initial phase of cyclization of squalene 2,3-oxide and its analogues.

That the bi-secondary oxides (I) and (II) are very much poorer substrates than either squalene 2,3-oxide (IV) or the homosqualene oxide analogue (VII) speaks for the overriding importance of a trisubstituted centre at C-2[7] and suggests an appreciable degree of S_N1 character in the reaction concerned with closure of ring A.

The cyclization of (I) but not of (II) may reflect (a) the presence of a region of the enzyme active site that is specific for interaction with the *trans*-1-methyl (4α-methyl in the developing steroid ring A), or (b) an unfavourable steric interaction of the *cis*-1-methyl (potential 4β-methyl of the sterol) with the 6-methyl (C-19 of the sterol) or with some other neighbouring structure in the enzyme active site.

The present result supports the view that of the two possibilities (b) (unfavourable steric interaction in the region of a 4β-alkyl substituent) must take precedence over (a) (specific enzyme interaction with a 4α-substituent). If, indeed, the 4α-substituent interacts with a receptor area in the active site, its specificity must be low enough to accommodate an ethyl group in place of methyl. That there is, however, some degree of specificity in the 4α-alkyl group interaction is suggested by the lower efficiency of cyclization of (VII) than of (IV) and by the reported[2] failure of cyclization of the (presumed) mixture of mono-ethyl substituted bi-secondary oxides (IX) and (X).

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[4] See ref. 6 and references cited therein.
[5] W. Voser, M. Montavon, H. H. Gunthard, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta*, 1950, **33**, 1893.
[6] K. J. Stone, W. R. Roeske, E. E. van Tamelen, and R. B. Clayton, preceding communication.
[7] No enzymic cyclization is observed with the 1,1′-bisnor-oxide (I; $R^1 = R^2 = H$); R. G. Nadeau, unpublished results. (See also ref. 2.)