Enzymic Transformation of an Acyclic Sesterterpene Terminal Epoxide into a Lanosterol Analogue

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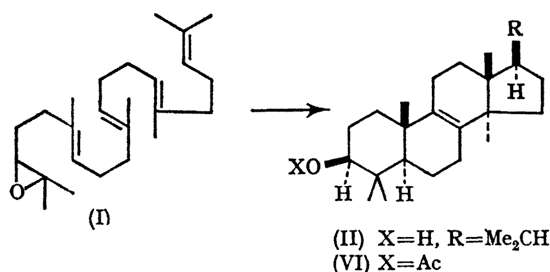
SUBSEQUENT to the finding that squalene 2,3-oxide is a general intermediate in the enzymic conversion of squalene into lanosterol and other 3-hydroxylated sterols, the transformation of structurally modified squalene oxides into sterol-like products was first reported from these laboratories.[1] In that preliminary study, the effect on side-chain reduction and shortening was tested and found not to deter seriously the biological cyclization process. In extending this approach, we have now determined that the absence of an entire isoprenoid unit from the non-oxidized terminus of squalene oxide does not prevent normal biochemical cyclization:



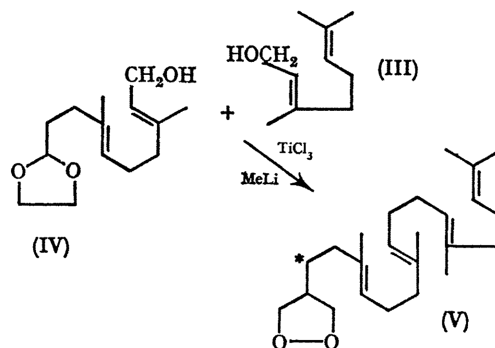
(II) X=H, R=Me_2CH
(VI) X=Ac

2,6,10,15,19-Pentamethyleicosa-*trans,trans,trans*-penta-2,6,-10,14,18-ene 2,3-oxide (I) gives rise to 23,24,25,26,27-pentanorlanost-8-en-3β-ol (II).

The desired substrate was prepared by the one-step, titanium(II) intermediated[2,3] coupling of geraniol (III) and the *trans,trans*-acetal (IV) (derived by degradation of farnesol)[3] followed by tritium labelling (*) of aldehyde corresponding to the tetraenyl acetal (V), and final re-formation of terminal epoxide, as previously described.[3] Epoxide (I), purified by t.l.c., was found to be a colourless oil, exhibiting an n.m.r. spectrum similar to that of squalene and consonant with the indicated structure.

Incubations were carried out with 100 μg ($9\cdot94 \times 10^6$ d./min.) of epoxide (I) dispersed by means of 100 μg. of Tween 80 in 10 ml. of the usual cyclase preparation[4] at 37° for 30 min. Work-up involved saponification with aqueous methanolic potassium hydroxide and extraction with hexane. After t.l.c. on silica gel, the product was shown by radioscanning to consist of two compounds in a 1:3 ratio and having R_F 0·36—0·48 (sterol region) and 0·54—0·74 (epoxide region). In a boiled enzyme control, the lower-R_F peak was much diminished.

A portion of the crude material from the sterol region was admixed with authentic (II) (see below) and co-recrystallized (four times from methanol) to constant specific activity (23% loss of original specific activity). The recrystallized alcohol was acetylated and the acetate was recrystallized five times from acetone, during which procedure the specific activity fell another 23% and became essentially constant. Another portion of the crude sterol product was silylated with trimethylsilyl chloride in pyridine and subjected to fractionation by preparative g.l.c. (5% diethyleneglycol succinate at 190°), 47% of the radioactivity appearing in a single peak having the same retention time ($R_{cholestane}$ 0·71) as the trimethylsilyl ether of authentic (II). The radioactive silyl ether was collected, hydrolysed with ethanolic KOH, and subjected to preparative g.l.c. on an XE-60 column at 180°; 91% of the activity was found in a fraction with the same retention time ($R_{cholestane}$ 1·90) as authentic (II).



An authentic sample of the enzymic product was made available by side-chain degradation of 25,26,27-trisnorlanosterol-24-carboxylic acid,[5] (II; R = $-CHMe\cdot CH_2\cdot CH_2\cdot CO_2H$), obtained by chromic acid oxidation of the corresponding aldehyde. By treatment of the acid with phenyllithium in tetrahydrofuran, followed by acetylation, the phenyl ketone (VI; R = $-CHMe\cdot CH_2\cdot CH_2\cdot COPh$), m.p. 180—182°, was obtained. Bromination in acetic acid–methylene chloride converted the aforementioned ketone into the α-bromo-ketone (VI; R = $-CHMe\cdot CH_2\cdot CHBr\cdot COPh$), m.p. 195·5—197°. The bromohydrin resulting from treatment of the bromo-ketone with sodium borohydride was not purified extensively or characterized, but directly ring-closed by means of potassium carbonate to the expected 23,24-oxide (VI; R = $-CHMe\cdot CH_2\cdot CH—CHO\cdot Ph$), m.p. 195—198°. Preparatory to side-chain cleavage, the expoxide was ring-opened by means of perchloric acid in aqueous tetrahydrofuran to the corresponding glycol, which was subjected, after saponification of the acetate unit, to the action of sodium metaperiodate in aqueous tetrahydrofuran. The resulting aldehyde after silica gel chromatography and crystallization from aqueous methanol, melted at 167—170°. Synthesis of the desired lanosterol variant (II) was achieved by decarbonylation of the aldehyde, carried out by treatment with tris(triphenylphosphine)rhodium chloride[6] in benzene. After chromatography on silica gel, the pentanor-sterol was recrystallized

from methanol–methylene dichloride. The authentic sterol melted at 191·0—191·5° and could be sublimed at 100° (0·08 mm).†

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† Except as noted, all intermediates were adequately characterized by analytical and spectral data.

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