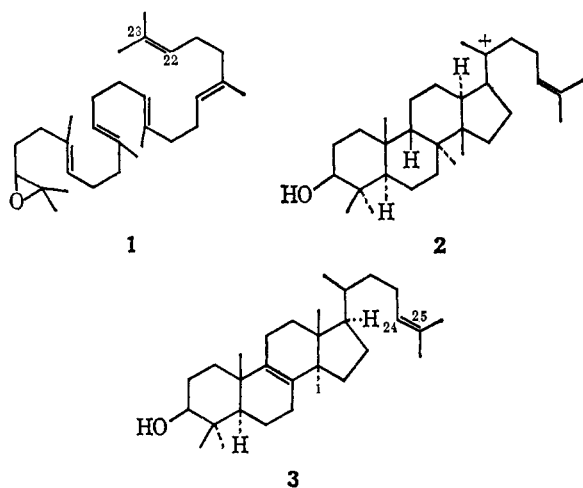


Formation of Sterols by the Action of 2,3-Oxidosqualene–Sterol Cyclase on the Factitious Substrates 2,3:22,23-Dioxidosqualene and 2,3-Oxido-22,23-dihydrosqualene

Sir:

Recent studies have shown the intermediacy of 2,3-oxidosqualene (**1**) in the biosynthesis of lanosterol under the influence of an enzyme which can be obtained in partially purified, water-soluble form.^{1–5} In the enzymic cyclization of the oxide **1** to the hypothetical protosterol stage (**2**) and the further rearrangement to lanosterol (**3**), one of the original ethylenic linkages survives. This reactive unit at C₂₂–C₂₃ is therefore available as a possible site for temporary covalent bonding to the enzyme during lanosterol biosynthesis. Such an interaction might be expected to assist directed cyclization by helping to impose upon **1** the proper folded conformation for concerted cyclization to **2**. The present note describes two independent tests which reveal that the Δ^{22} olefinic linkage is *not essential* to sterol formation as effected by 2,3-oxidosqualene–sterol cyclase and which, therefore, argue against its chemical involvement. In one set of experiments 2,3:22,23-dioxidosqualene (**4**) was used as substrate and in the



other 2,3-oxido-22,23-dihydrosqualene (**5**) was employed. Anaerobic incubation with particle-free solutions of 2,3-oxidosqualene–sterol cyclase⁸ resulted in conversion of the dioxide **4** to 24,25-oxidolanosterol and the monooxide **5** to 24,25-dihydrolanosterol.

The product from the ¹⁴C-labeled dioxide⁶ **4** was isolated after a 20-hr incubation period at 37° by successive saponification, extraction, and thin layer chromatography (tlc) on silica gel (CH₂Cl₂ development). Approximately 25% of the total radioactivity appeared

(1) E. J. Corey, W. E. Russey, and P. R. Ortiz de Montellano, *J. Am. Chem. Soc.*, **88**, 4750 (1966).

(2) E. J. Corey and W. E. Russey, *ibid.*, **88**, 4751 (1966).

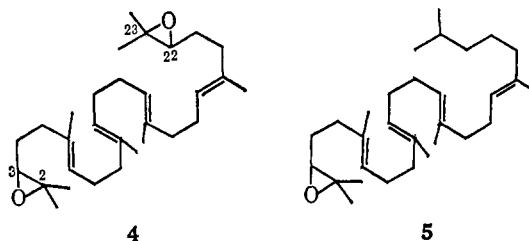
(3) P. D. G. Dean, P. R. Ortiz de Montellano, K. Bloch, and E. J. Corey, *J. Biol. Chem.*, **242**, 3014 (1967).

(4) E. J. Corey, P. R. Ortiz de Montellano, K. Lin, and P. D. G. Dean, *J. Am. Chem. Soc.*, **89**, 2797 (1967).

(5) E. E. van Tamelen, J. D. Willet, R. B. Clayton, and K. E. Lord, *ibid.*, **88**, 4752 (1966).

(6) Labeled dioxide **4** was synthesized starting with [¹⁴C]squalene, which had been prepared from labeled mevalonic acid in a rat liver homogenate, *via* the dibromohydrin [see E. E. van Tamelen and T. J. Curphey, *Tetrahedron Letters*, 121 (1962)]. Since the synthetic dioxide **4** obtained by this process (isolated by preparative layer chromatography) was not separated into isomeric forms, it must consist of both *dl* and *meso* isomers, probably in statistical distribution.

in a band, *R_f* 0.14, corresponding to that of authentic 24,25-oxidolanosterol, mp 107–109°,⁷ and different from



that of lanosterol, *R_f* 0.23. Admixture of the labeled product with nonlabeled 24,25-oxidolanosterol and successive recrystallization from aqueous methanol led to material of essentially constant specific radioactivity over four cycles of recrystallization. The identity of the enzymic transformation product of **4** was further investigated by acetylation of the labeled sterol fraction. The radioactive acetate so produced was chromatographically identical with authentic 24,25-oxidolanosteryl acetate and different from lanosteryl acetate. In addition, reduction of the labeled sterol fraction with lithium aluminum hydride led to a product which was chromatographically identical with 25-hydroxylanosterol (mp 177–180°, synthesized similarly by reduction of authentic 24,25-oxidolanosterol with LiAlH₄). Recrystallization of a mixture of this hydride-reduced sterol fraction and 25-hydroxylanosterol led to constant specific radioactivity over four recrystallizations. These data demonstrate that the dioxide **4** is converted enzymically to sterol, specifically 24,25-oxidolanosterol.^{8,9}

The radioactive product from anaerobic incubation (1 hr) of 2,3-oxido-22,23-dihydrosqualene (**5**), labeled at C₂₂ with tritium,¹⁰ with a solution of 2,3-oxidosqualene–sterol cyclase corresponded chromatographically (tlc) to authentic 24,25-dihydrolanosterol (*ca.* 20% conversion). Acetylation of this product afforded an acetate which was shown to be chromatographically identical with authentic 24,25-dihydrolanosteryl acetate and different from lanosteryl acetate by tlc using silver nitrate impregnated silica gel¹¹ and by vpc analysis on a column of 2% Epon 1001 on Diatoport S.¹ Further, recrystallization of this acetylated product with added unlabeled 24,25-dihydrolanosteryl acetate led to con-

(7) Prepared from lanosterol by hydroxybromination with N-bromosuccinimide in aqueous glyme and subsequent treatment of the resulting 24,25-bromohydrin (purified chromatographically) with sodium isopropoxide–isopropyl alcohol, and probably a mixture of co-crystallizing C₂₅ epimers.

(8) In a note which appeared during the preparation of this manuscript E. E. van Tamelen, J. D. Willet, and R. B. Clayton, *J. Am. Chem. Soc.*, **89**, 3371 (1967), report that "labeled lanosterol was not detected after incubation of ³H-labeled squalene 2,3:22,23-dioxide with rat liver homogenate" (italics ours). If their experiments *did* reveal cyclization of **4**, they failed to indicate this finding.

(9) The enzymic cyclization of **4** is several times slower than that of 2,3-oxidosqualene (**1**) as might be expected for an "unnatural" substrate. Since a conversion of **4** to oxidolanosterol of *ca.* 50% can be achieved by two cycles of incubation, it appears that the configuration at C₂₂ in the dioxide **4** is not critical to cyclization.

(10) Labeled **5** was synthesized from **1** by a sequence consisting of hydrolysis of the oxide to the corresponding glycol, oxidation (HIO₄) to a C₂₇ aldehyde, reaction with isopropyllithium to give 2-hydroxy-2,3-dihydrosqualene, oxidation (CrO₃–pyridine) to the corresponding ketone, conversion to the *p*-toluenesulfonylhydrazone, reduction to 2,3-dihydrosqualene using ³H-labeled sodium borohydride [see L. Caglioti and P. Grasselli, *Chem. Ind. (London)*, 153 (1964)], hydroxy bromination, and cyclization of the bromohydrin to epoxide.¹

(11) As F. C. den Boer [*Z. Anal. Chem.*, **205**, 308 (1964)] has shown, this column resolves lanosteryl and 24,25-dihydrolanosteryl acetates.

stant specific radioactivity over three cycles after the initial recrystallization. This evidence clearly indicates that 2,3-oxido-22,23-dihydrosqualene is cyclized enzymically to 24,25-dihydrolanosterol. In addition, it has been found that ^3H -labeled 2,3-dihydrosqualene¹⁰ upon aerobic incubation with rat liver homogenate affords a radioactive product which is chromatographically identical with cholesterol.

The enzymic cyclization of **5** occurs at a rate which is comparable to that for 2,3-oxidosqualene (**1**). By comparison, the cyclization of the dioxide **4** is considerably slower.

If it can be assumed that the enzyme responsible for the cyclization of **4** and **5** is that which cyclizes 2,3-oxidosqualene, as seems highly probable, that enzyme does not interact covalently with the Δ^{22} double bond of **1**. Further studies are in progress to determine whether other structurally modified analogs of **1** can be cyclized to sterol systems under the influence of 2,3-oxidosqualene-sterol cyclase. It is noteworthy that 9,10-dihydrosqualene is epoxidized but not cyclized in rat liver homogenate.^{2,12,13}

(12) This work was supported in part by the National Institutes of Health.

(13) We are grateful to P. R. Ortiz de Montellano, K. Lin, and P. D. G. Dean for valuable assistance during the course of this study.

(14) Radcliffe Institute Scholar, 1966-1967.

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The Structure of Illudol, a Sesquiterpenoid Triol from *Clitocybe illudens*

Sir:

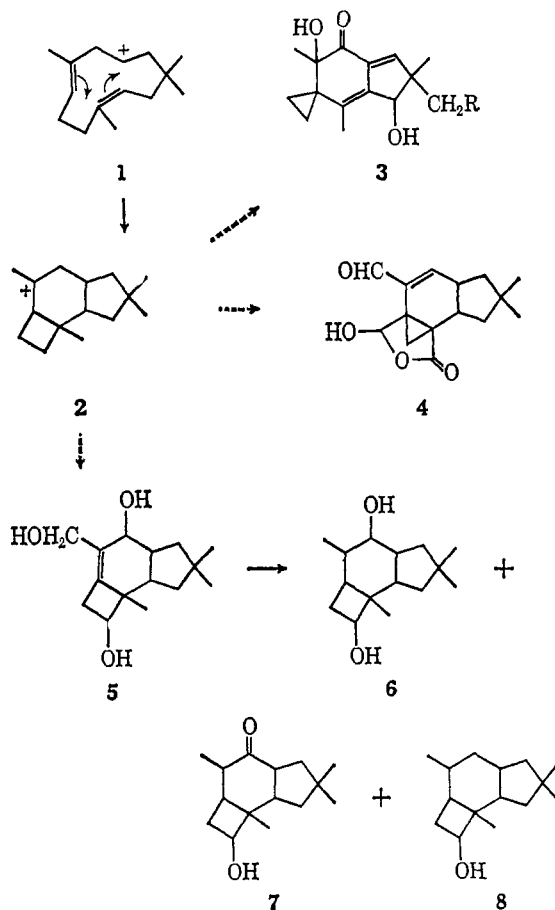
We recently proposed a biogenetic scheme for the formation of the sesquiterpenoids illudin-S (**3**, R = OH) and -M (**3**, R = H). This involved a humulene-type precursor **1** and the tricyclic cation **2**.¹ A similar route has also been proposed for the genesis of marasmic acid (**4**).² We have now found that a third metabolite of *Clitocybe illudens*, "illudol" (the "inactive compound"³), has structure **5**, with the same carbon skeleton as the postulated precursor (**2**) of the illudins.

Illudol, mp 130-132°, $[\alpha]_D -116^\circ$ (*c* 0.42, 95% ethanol), analyzed for $\text{C}_{15}\text{H}_{24}\text{O}_3$, formed a triacetate, and was monounsaturated (λ_{max} 207 $\text{m}\mu$ (ϵ 9400)), and hence tricyclic. The mass spectrum did not show the molecular ion peak (mol wt 252) but gave peaks at *m/e* 234 (*M* - 18) and 216 (*M* - 2(18)). The nmr spectrum showed signals for three tertiary methyl groups (τ 9.03, 9.0, and 8.92) and a singlet at τ 5.78 (2 H) partly overlapping multiplets due to two other protons. On acetylation, this singlet was shifted to τ 5.45, confirming the presence of a primary hydroxyl group, possibly allylic. Absence of any other low-field signals indicated that the double bond was tetrasubstituted. Signals in the triacetate at τ 5.37 (1 H, triplet) and 4.7 (1 H, poorly resolved doublet) indicated

(1) T. C. McMorris and M. Anchel, *J. Am. Chem. Soc.*, **87**, 1594 (1965).

(2) J. J. Dugan, P. de Mayo, M. Nisbet, J. R. Robinson, and M. Anchel, *ibid.*, **88**, 2838 (1966).

(3) M. Anchel, A. Hervey, and W. J. Robbins, *Proc. Natl. Acad. Sci. U. S.*, **36**, 300 (1950).



that there were two secondary hydroxyl groups in the parent compound.

Catalytic hydrogenation of **5** with palladium on charcoal gave a complex mixture from which three crystalline compounds were isolated by chromatography on silica gel. One was a diol (**6**), $\text{C}_{15}\text{H}_{26}\text{O}_2$, mp 94-95°. Its nmr spectrum integral showed the presence of four methyl groups (signals at τ 9.09, 8.99, and 8.95), indicating that hydrogenolysis of the primary hydroxyl group had taken place. In agreement, there were now only two protons α to oxygen, *viz.*, at τ 6.6 (broad peak) and 5.47 (triplet), shifted to τ 5.09 and 4.67, respectively, on addition of CCl_3CONCO . The second product was a keto alcohol (**7**), mp 110-112° (mol wt 236), ν_{max} 3290 and 1709 cm^{-1} . The formation of this compound by hydrogenolysis of the primary hydroxyl group and migration of the double bond in **5** indicated that one of the secondary hydroxyl groups in **5** was allylic and present in a six-membered ring. The third product of hydrogenation, mp 87-91°, ν_{max} 3280 cm^{-1} , was the monoalcohol **8**.

Treatment of illudol with palladium on charcoal at 280° gave a mixture of hydrocarbons. The major component, isolated by vapor phase chromatography, was identical with 2,2,4,5,6-pentamethylindan² (ultra-violet, infrared, nmr). Formation of a C_{14} bicyclic aromatic compound from illudol on dehydrogenation, considered in conjunction with the above evidence, suggested that the third ring of this tricyclic triol was four membered. In confirmation, oxidation of **6** and **7** with Jones reagent⁴ gave a diketone, ν_{max} 1782 (cyclobuta-

(4) A. Bowers, T. G. Halsall, E. R. H. Jones, and A. J. Lemm, *J. Chem. Soc.*, 2548 (1953).