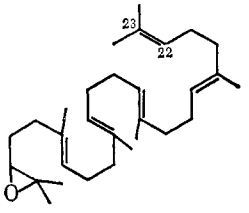
Formation of Sterols by the Action of 2,3-Oxidosqualene–Sterol Cyclase on the Factitious Substrates 2,3:22,23-Dioxidosqualene and 2,3-Oxido-22,23-dihydrosqualene

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 C_{22}–C_{23} 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



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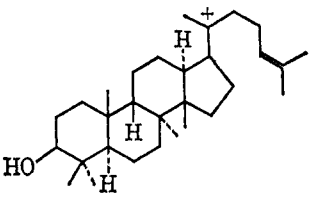

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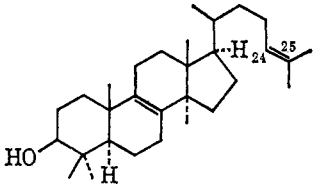

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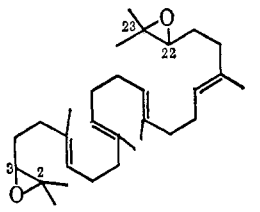
other 2,3-oxido-22,23-dihydrosqualene (**5**) was employed. Anaerobic incubation with particle-free solutions of 2,3-oxidosqualene–sterol cyclase[8] resulted in conversion of the dioxide **4** to 24,25-oxidolanosterol and the monooxide **5** to 24,25-dihydrolanosterol.

The product from the ^{14}C-labeled dioxide[6] **4** was isolated after a 20-hr incubation period at 37° by successive saponification, extraction, and thin layer chromatography (tlc) on silica gel (CH_2Cl_2 development). Approximately 25% of the total radioactivity appeared in a band, R_f 0.14, corresponding to that of authentic 24,25-oxidolanosterol, mp 107–109°,[7] and different from



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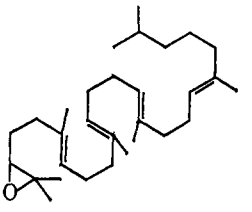

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that of lanosterol, R_f 0.23. Admixture of the labeled product with nonlabeled 24,25-oxidolanosterol and successive recrystallization from aqueous methanol led to material of essentially constant specific radioactivity over four cycles of recrystallization. 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 $LiAlH_4$). 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 C_{22} 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 gel[11] 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-

(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 [^{14}C]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.

(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 cocrystallizing C_{25} 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 C_{22} 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 (HIO_4) to a C_{27} aldehyde, reaction with isopropyllithium to give 2-hydroxy-2,3-dihydrosqualene, oxidation (CrO_3–pyridine) to the corresponding ketone, conversion to the *p*-toluenesulfonylhydrazone, reduction to 2,3-dihydrosqualene using ^3H-labeled sodim 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 ^{3}H-labeled 2,3-dihydrosqualene[10] 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]

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(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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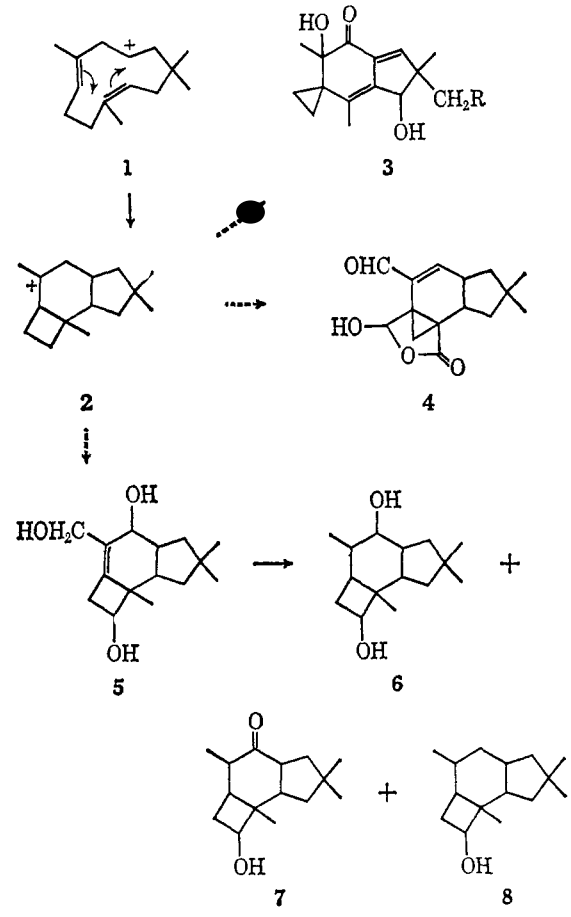

The Structure of Illudol, a Sesquiterpenoid Triol from *Clitocybe illudens*

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° (*c* 0.42, 95% ethanol), analyzed for $C_{15}H_{24}O_3$, formed a triacetate, and was monounsaturated (λ_{max} 207 mμ (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 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**), $C_{15}H_{26}O_2$, 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-pentamethylindan[4] (ultraviolet, 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 reagent[4] gave a diketone, ν_{max} 1782 (cyclobuta-

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