(X = adenylate) is inactive in enzymatic light production.

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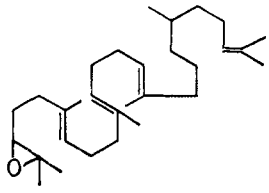
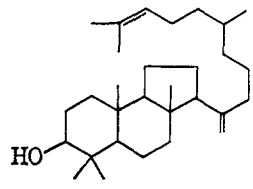
Enzymic Cyclization of *trans,trans,trans*-18,19-Dihydrosqualene 2,3-Oxide

Sir:

With the hope of shedding light on the cyclization stage of lanosterol biosynthesis, we have carried out enzymic experiments on an appropriately modified substrate, [4-^{3}H]*trans,trans,trans*-18,19-dihydrosqualene 2,3-oxide (I).[1,2] We find that the rat liver enzyme preparation which effects the normal biosynthesis of



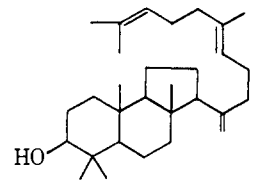
I



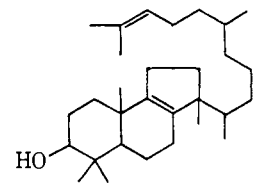
II(A,B)

lanosterol from squalene 2,3-oxide also converts in reasonable yield this dihydrosqualene oxide to the perhydrocyclopenta[*a*]naphthalene derivative A (gross structure II), representing a skeletal type previously encountered as a nonenzymic product (III) of squalene oxide cyclization.[4]

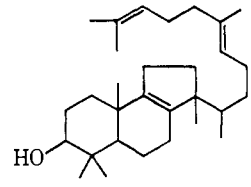
Enzymic reaction of the radiolabeled 18,19-dihydrosqualene 2,3-oxide (I) was carried out in a clarified (110,000*g* supernatant) preparation of 2,3-oxidosqualene–lanosterol cyclase isolated from the microsomal fraction of rat liver.[5] In an exemplary run, 1.179 mg of oxide (60.00×10^6 dpm; specific activity = 51,300 dpm/μg) was incubated for 4 hr at 37° under nitrogen with 65 ml of enzyme preparation equivalent to 45 g of rat liver. The radioactive material (54.76×10^6 dpm), following saponification and extraction, was separated by tlc[7] to yield as a major product (~8% yield) a substance A (R_f 0.41; lanosterol gave R_f 0.37). By these means, we accumulated a total of 220 μg of product A on which the experiments described below were carried out. For comparison purposes, nonenzymic cyclization products B (gross structure II) and C and D (gross structure IV) were prepared from 18,19-dihydrosqualene 2,3-oxide (I) by means previously utilized for the production of the analogous tricycles III[4] and V [(C′)[4] and (D′)[8]] from squalene 2,3-oxide.



III



IV (C, D, E, F)



V (C′, D′)

By means of a 100-Mc Varian instrument, there was obtained a time-averaged nmr spectrum of enzymic product A (220 μg in CCl_4) which displayed the following peaks (values relative to TMS = 10.0): 4.92 (1 H, triplet), >C=CH–; 5.17 (1 H, singlet) and 5.42 (1 H, singlet), >C=CH_2; 6.85 (1 H, triplet), axial >CHO(H); 8.35 (3 H, singlet) and 8.42 (3 H, singlet), two >C=C-(CH_3)–; and 9.05, 9.10, 9.16, and 9.27 (*ca.* five methyls on saturated carbon). Essentially identical resonances for comparable protons were observed in a 60-Mc nmr spectrum of III.[4]

The mass spectra of the TMSE derivatives of the tricyclic alcohols A and B display fragmentation patterns[9] which differ *only* in the relative intensity of certain peaks; *cf. m/e* 189, 190, 191 [$C_{14}H_{21-23}$], and 229 [$C_{17}H_{25}$] which are characteristic of this carbon skeleton and consistent with gross structure II and are in accordance with analogous assignments and conclusions in the squalene series (structure III). Although possessing identical R_f values (0.41) on tlc, substances A (glpc, R_c = 1.60) and B (glpc, R_c = 1.91) exhibit glpc retention times in the ratio 0.83:1.[10] Certain relative peak intensity differences in these otherwise identical fragmentation patterns and their glpc behavior indicate structural formulation of compounds A and B as stereoisomers.

In order to confirm structural assignment II to sub-

(1) Synthesis: E. E. van Tamelen, K. B. Sharpless, and R. Hanzlik, *J. Am. Chem. Soc.*, in press.

(2) Enzymic conversion of squalene and squalene 2,3-oxide variants (22,23-dihydro and 23,24,24′-trisnor) to lanosterol-like systems was first disclosed by E. E. van Tamelen, K. B. Sharpless, J. D. Willett, R. B. Clayton, and A. L. Burlingame, *ibid.*, **89**, 3920 (1967). The 22,23-dihydrosqualene oxide case was duplicated by E. J. Corey and S. K. Gross, *J. Am. Chem. Soc.*, **89**, 4561 (1967), who reported in addition the enzymic transformation of squalene 2,3:22,23-dioxide[3] to lanosterol 24,25-oxide.

(3) For preparation, see E. E. van Tamelen and T. J. Curphey, *Tetrahedron Letters*, 121 (1962).

(4) E. E. van Tamelen, J. D. Willett, M. Schwartz, and R. Nadeau, *J. Am. Chem. Soc.*, **88**, 5937 (1966).

(5) The method used for this preparation was a modification of that indicated by Dean, *et al.*[6] Its properties will be described elsewhere.

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

(7) All thin layer R_f values refer to mobilities on unactivated silica gel G plates which were eluted with a solution of 20% ethyl acetate in hexane.

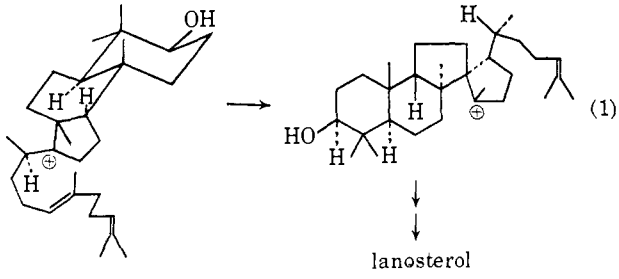
(8) D′ (V) is a new tricyclic alcohol which was recently isolated from the chemical cyclization which yields the known tricycle C′ (V). The mass spectra and nmr spectra of D′ and of its 18,19-dihydro analog D indicate that these compounds are stereoisomeric with the known C′ and C structures, respectively (unpublished results, K. B. Sharpless).

(9) These were determined under identical conditions assuring quantitatively reproducible fragmentation patterns using an A.E.I. MS-12 mass spectrometer.

(10) Glpc data were obtained for trimethylsilyl ether on terminated Carbowax (5%) ("steroid analytical phase," Wilkins Instrument Co.) on Chromosorb W at 218° with a nitrogen flow rate of 90 cc/min; R_c = retention time relative to cholestane.

stances A and B, each was converted by a Lewis acid promoted process to the skeletally isomeric system of type IV, the counterpart of established structure V.[2] Enzyme product A, on being subjected to the action of stannic chloride in benzene at room temperature for 25 min, gave rise to a 7:3 mixture of two isomers, E (glpc, R_c = 1.64) and F (glpc, R_c = 1.15). Under similar conditions, nonenzymic product B was transformed into a pair of isomers, C (glpc, R_c = 1.34) and D (glpc, R_c = 0.81) in the ratio 3:2. As indicated by glpc and mass spectral comparisons (on TMSE), substances C and D were identical with the pair of tricyclic alcohols also obtained by direct cyclization of dihydrosqualene oxide I. Products E and F exhibited mass spectra indistinguishable from each other and also from those of authentic materials C and D; furthermore, the mass spectra of the free alcohols C, D, E, and F were identical. Like V, all the dihydro relatives (as TMSE) gave mass spectral fragmentation patterns with a base peak at *m/e* 229. Like the relationship between compounds A and B, the differences between the isomers of IV (C, D, E, and F) are considered to be stereochemical and are under investigation in these laboratories.

This highly interesting result represents the first reported case of the generation by the 2,3-oxidosqualene–lanosterol cyclase system of a cyclic structure different from that of lanosterol. The significance of this finding cannot be fully assessed at present.[11] It is pertinent to point out that in a new, alternative interpretation (eq 1)



(1)

of the chemistry of lanosterol biosynthesis, a perhydrocyclopenta[*a*]naphthalene appears as an initial cyclization product which then produces the lanosterol skeleton *via* a subsequent [5.5]spiro intermediate. Whether enzymic product A is generated by an aberrant biosynthetic route or whether it reflects the normal—albeit interrupted—enzymic pathway remains for future investigations to demonstrate. The obvious next stage, preparation and enzymic testing for sterol formation of appropriate perhydrocyclopenta[*a*]naphthalenes, is now being entered in these laboratories.

Acknowledgment. This work was supported by Grants GM 10421 (to E. v. T.) and GM 12493 (to R. B. C.) from the National Institutes of Health, and by a grant-in-aid from the American Heart Association (to R. B. C.). Contributors from the University of California at Berkeley acknowledges financial support from the National Aeronautics and Space Administration (Grants NsG 101 and NGR 05-003-134). We wish to thank Mr. Fred C. Walls for technical assistance in determining the mass spectra (University of California, Berkeley) and Dr. Toshaiki Nishida (Stanford University) for the time-averaged nmr spectra.

(11) A plant product presumably biogenetically related to squalene has also recently been observed to possess the perhydrocyclopenta[*a*]-naphthalene skeleton (personal communication from Dr. S. Dev, National Laboratory, Poona, India).

(12) National Institutes of Health Predoctoral Fellow, 1965–1967.
(13) National Science Foundation Predoctoral Fellow, 1966–1968.

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The Effect of Active Ester Components on Racemization in the Synthesis of Peptides by the Dicyclohexylcarbodiimide Method[1]

Sir:

Following the report by Wünsch and Drees[2] of a favorable effect of N-hydroxysuccinimide (HOSu) on yields in a peptide synthesis by the N,N′-dicyclohexylcarbodiimide (DCCD) procedure, we began a study of the effect of HOSu on racemization in test syntheses by the same method. Weygand and associates[3,4] have since found a favorable effect in test syntheses using more than 1 equiv of HOSu but minimum requirements and mechanisms were not clear. We present evidence here that formation of intermediate HOSu ester of the acyl peptide is the major mechanism, and only 1 equiv of HOSu is required.

We reported some time ago[5] that 8% DL and 74% L tripeptide fractions were isolated in the reaction of Z-Gly-Phe-OH with H-Gly-OEt with DCCD at room temperature in tetrahydrofuran solvent. A repetition of the experiment with 1.1 equiv of HOSu present resulted in 0% DL and 90% L tripeptide. Using the more sensitive Young test,[6,7] whch involves the reaction of Bz-Leu-OH with H-Gly-OEt to form Bz-Leu-Gly-OEt, we found 79% DL, 0% L product by a room-temperature reaction with 1 equiv of DCCD only. When 1 equiv of HOSu was also present, the results were reversed: 0% DL- and 73% L-Bz-Leu-Gly-OEt were isolated. To test whether or not the mechanism involves neutralization of the basicity of DCCD, experiments with added pivalic acid were done. From the Z-Gly-Phe-Gly-OEt test synthesis, with 1 equiv of pivalic acid present, 0% DL and 80% L products were found. However, the Bz-Leu-Gly-OEt system yielded 75% DL and 0% L products. We conclude that neutralization of the basicity of DCCD is a minor mechanism, and formation of intermediate HOSu esters is the major path.

In several experiments, Bz-Leu-OSu was isolated by concentration of the filtrate *in vacuo* after removal of

(1) Presented at the 154th National Meeting of the American Chemical Society, Chicago, Ill., 1967, Abstract S-031.
(2) E. Wünsch and F. Drees, *Chem. Ber.*, **99**, 110 (1966).
(3) F. Weygand, D. Hoffmann, and E. Wünsch, *Z. Naturforsch.*, **21b**, 426 (1966).
(4) F. Weygand and U. Ragnarsson, *ibid.*, **21b**, 1141 (1966).
(5) G. W. Anderson and F. M. Callahan, *J. Am. Chem. Soc.*, **80**, 2902 (1958).
(6) M. W. Williams and G. T. Young, *J. Chem. Soc.*, 881 (1963).
(7) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Am. Chem. Soc.*, **88**, 1338 (1966).