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CORRELATION OF A PROTOSTEROL FROM 20,21-DEHYDRO-2,3-OXIDOSQUALENE AND 2,3-OXIDOSQUALENE-STEROL CYCLASE WITH DIHYDROLANOSTEROL

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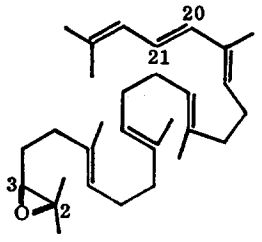
The artificial substrate 20,21-dehydro-2,3-oxidosqualene (I) is transformed by the enzyme 2,3-oxidosqualene-sterol cyclase (1) into a cyclization product for which the protosterol formulation II was indicated by chemical and physical data (2). The designation of stereochemistry for the tetracyclic part was based upon the assumption that the cyclizing enzyme exercises the same stereochemical control with the 20,21-dehydro derivative I as with 2,3-oxidosqualene itself. Although this premise seems reasonable, independent evidence of stereochemistry is clearly desirable; this has now been obtained in the following way.

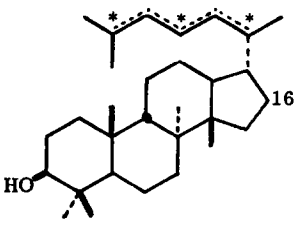
A sample of II labelled with tritium at C-16 was obtained from labelled I as previously described (2) using a crude solution of 2,3-oxidosqualene-sterol cyclase from hog liver. Partial purification of II was effected by thin layer chromatography (t.l.c.) (neutral alumina support) to remove most of the nonradioactive lipids originating in the crude enzyme preparation. Hydrogenation of II (1.8×10^5 d.p.m., 1.44 nanomoles) in 10 ml. of ethyl acetate using 5 mg. of 5% rhodium-on-charcoal catalyst (3) yielded a saturated product (1.1×10^5 d.p.m., 0.84 nanomole, 60%) having the same R_f as II (0.36) by t.l.c. analysis using 20% ethyl acetate in benzene with silica gel adsorbant. Acetylation of a part of this material (2.7×10^4 d.p.m.) using excess acetic anhydride--pyridine gave an acetate which was admixed with 50 mg. of unlabelled 24,25-dihydrolanosteryl acetate (IV) (4). A single recrystallization of the mixture from ethanol--water led to the isolation of nonradioactive crystalline dihydrolanosteryl acetate, thus indicating the absence of labelled dihydrolanosterol in the hydrogenation product derived from II. On the other hand, dihydrolanosteryl acetate was formed from perhydro II by acetylation after acid treatment following a procedure developed by Prof. D. Arigoni and coworkers for the rearrangement of 3β-acetoxy-4β-methyl-$\Delta^{13,17}$-fusidene to dihydrolanosteryl acetate (5).

The reduction product from II (5.4×10^4 d.p.m., 0.42 nanomole) was heated for 3 hr. at 90-100° with 10 ml. of acetic acid solution containing 30% by volume of conc. hydrochloric acid, and the material isolated therefrom was converted to the acetate using excess acetic anhydride--pyridine. Admixture of the labelled acetate so obtained (3.5×10^4 d.p.m., 0.27 nanomole) with 50 mg. of dihydrolanosteryl acetate and recrystallization from ethanol--water led to crystalline acetate having specific radioactivity (d.p.m./mg.) of 41.7, 36.2, 18.9, 18.7, and 18.8 for recrystallizations 1, 2, 3, 4, and 5, respectively.

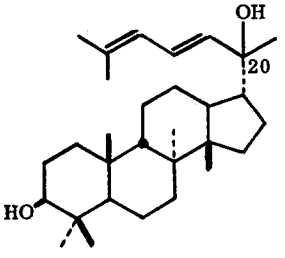
The formation of dihydrolanosteryl acetate from II by the three-step sequence--hydrogenation, acid treatment, acetylation--indicates that at least part of the enzymic cyclization product II can be formulated more specifically as having the hydroxyl function at C-20. Further, the fusidane stereochemistry for the tetracyclic nucleus, as shown in III, receives independent support (6).



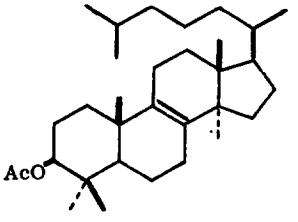
I



II, OH at one C*



III



IV

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1. P. D. G. Dean, P. R. Ortiz de Montellano, K. Bloch, and E. J. Corey, J. Biol. Chem., 242, 3014 (1967).
2. E. J. Corey, K. Lin, and H. Yamamoto, J. Amer. Chem. Soc., 91, 2132 (1969).
3. This catalyst was chosen to minimize hydrogenolysis of the allylic hydroxyl function; see, for example, P. N. Rylander, "Catalytic Hydrogenation over Platinum Metals," Academic Press, New York, 1967, p. 84.
4. An authentic sample, m.p. 120-121°, was obtained by the reduction of lanosterol with 1 equivalent of hydrogen with platinum catalyst in ethanol--ethyl acetate (5:1) followed by acetylation; see, L. Ruzicka, E. Rey, and A. C. Muhr, Helv. Chim. Acta, 27, 472 (1944).
5. We are indebted to Prof. Arigoni for supplying this procedure (May 1968).
6. This work was supported by the National Science Foundation.