Anal. Calcd for $C_{13}H_{18}NClO_4$: C, 54.24; H, 6.31. Found: C, 54.21; H, 6.24.

Resolution of 8-Deltacyclanone (5). Perchlorate salt **24a** (250 g, 0.87 mol) and potassium *d*-camphor-10-sulfonate (236 g, 0.87 mol) were added to methanol (500 ml) and the mixture was heated to reflux. After cooling the solution in a refrigerator overnight the potassium perchlorate was removed by filtration and the methanol removed on a rotary evaporator. The resulting oil was allowed to stand for 3 months. Crystals finally began to appear at this time. These were removed and the mother liquor was added to 200 ml of tetrahydrofuran. Cooling this solution caused several crops of crystals to be deposited. Recrystallization of these fractions gave products which had melting points ranging from 63 to 108° and optical rotations from +0.19 to +2.94° (1 g/10 ml of methanol, 1-dm cell, sodium D line). After repeated attempts to purify these crystals, the fractions having a rotation of +1.8° and greater were finally combined and hydrolyzed by adding them to a 20% aqueous potassium hydroxide solution. Extraction of these solutions three times with ether, drying ($MgSO_4$), evaporation of the ether, and distillation gave 25 g of tetracyclic ketone **5** having $[\alpha]^{24}_{304}$ −90° (*c* 0.05, methanol).

Kinetic Measurements. The chosen acetolysis conditions and procedures were similar to those of Tanida, *et al.*[31] Rate constants were determined at 25.68 ± 0.02° using a nonlinear least squares curve fitting program (E-2-OSU-CURV FIT). The determination of the rate constant for **1**-OBs, $k_{exo} = 2.61 \pm 0.09 \times 10^{-4}$ sec^{-1}, is based upon three runs and that for **2**-OBs, $k_{endo} = 4.62 \pm 0.06 \times 10^{-6}$, is based upon two runs.

Acknowledgments. The authors gratefully acknowledge the generous support of this work by the National Science Foundation, the Air Force Office of Scientific Research, and the University of Idaho Research Council, and express their thanks to Mr. Lewis Cary, Varian Associates, Palo Alto, Calif., and Dr. Jerry Heeshan, Chemical Physics Laboratory, Dow Chemical Co., Midland, Mich., for 220- and 100-MHz nmr spectra.

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The Nature of the Hydrogen Migrations in the Cyclization of Squalene Oxide to Lanosterol

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Received July 12, 1969

Abstract: The hydrogen migrations that occur during the conversion of squalene to lanosterol have been shown to take place by a sequence of two 1,2-shifts of hydrogen and not as a single 1,3-hydrogen shift. Squalene labeled with tritium at C-14 was converted to lanosterol with a rat liver homogenate; no tritium is located at C-20 of the resultant lanosterol, whereas the anticipated amount of tritium is found at C-17. Thus, the tritium originally at C-14 of squalene becomes attached to C-17 of the lanosterol, as the result of a sequence of two 1,2-shifts of hydrogen.

The conversion of squalene to lanosterol[1-3] has recently been shown to involve oxidation of squalene to 2,3-oxidosqualene and then cyclization of this substance to lanosterol[4-8] with retention of the oxygen of the epoxide function as the C-3 hydroxyl of lanosterol.[9] No cofactor requirements for the cyclase enzyme could be demonstrated.[9,10]

The folding of squalene suggested in 1953[11,12] (Scheme I) and subsequently amplified to include the stereochemical features of triterpene substances[13-15] has been amply confirmed by experiments. In particular, the sequence of 1,2-methyl migrations postulated to occur during the rearrangement of a protosterol cation to lanosterol has been demonstrated.[16-18] However, the nature of the hydrogen migrations are still unsettled. A study of the fate of tritium during the conversion of (4*R*)-mevalonic-4-^{3}H acid to cholesterol has been reported;[19] however, this work does not distinguish between a single 1,3-hydrogen shift, for which there is ample precedence in organic chemistry,[20-24] and a se-

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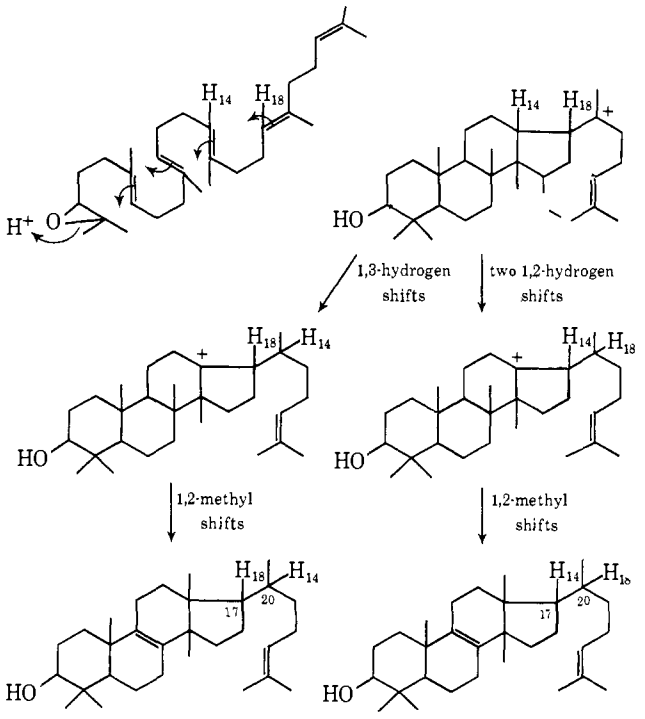
Scheme I



quence of two 1,2-migrations as postulated. With (4*R*)-mevalonic-4-^{3}H acid as substrate, tritium will be present at both C-17 and C-20 of cholesterol whether a single 1,3-hydrogen migration or two 1,2-shifts occur during the transformation of the squalene to the lanosterol skeleton. This cyclization is shown in Scheme I where the consequences of two 1,2-hydrogen migrations or a single 1,3-hydrogen shift are outlined.

Recently an alternate mechanism for cyclization of squalene has been proposed[6] (see Scheme II) which actually involves a 1,3-hydrogen migration. The hydrogen originally at C-18 of squalene ends up at C-20 of lanosterol and the hydrogen at C-14 of squalene is predicted to become attached to C-17 of lanosterol. This proposal thus results in the same predicted origins of the hydrogens as the earlier ones involving a sequence of 1,2-hydrogen migrations.

Attention has also been drawn to the possible separation of cyclization and rearrangement steps. The possibility of an intermediate with an acquired nucleophile (either from the enzyme or from solution) was first suggested in 1964,[25] and also has been raised by others.[26] Recently, evidence has been accumulated[27,28] that the steps from squalene oxide (I) lanosterol (III) may not be completely concerted but that there may be a pause after formation of the tetracyclic nucleus. After rotation about the C-17, C-20 bond, rearrangements, including the hydrogen migrations that are the subject of this paper, occur to convert the protosterol intermediate (II) into lanosterol (III) as shown in Scheme III.

The work described in this paper shows unambiguously that the migrations of hydrogen are indeed 1, 2; *i.e.*, tritium from C-14 squalene is found only at C-17 of lanosterol.

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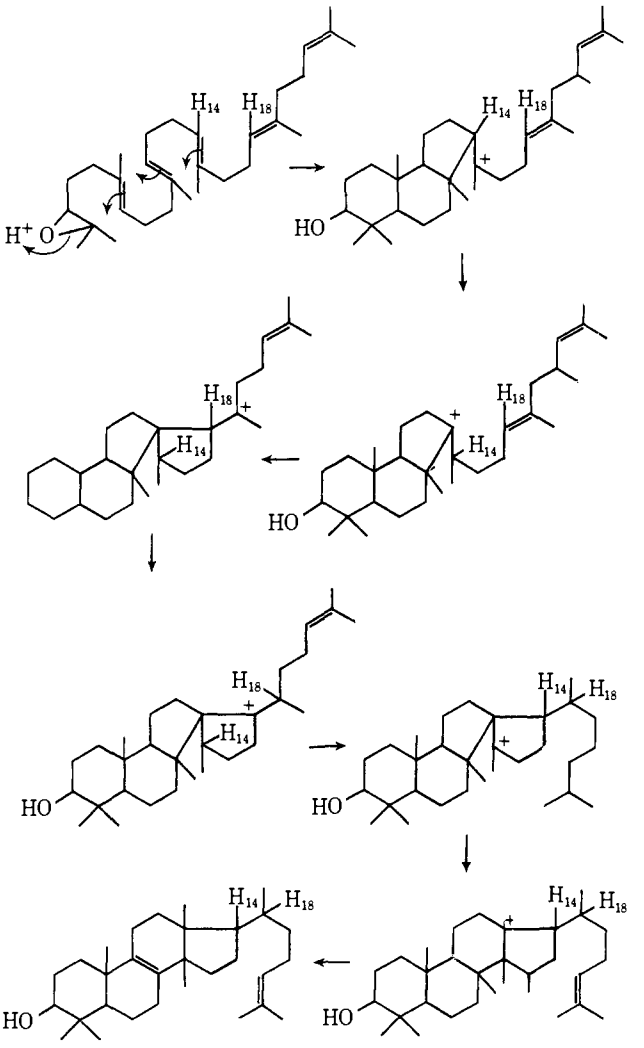
Scheme II



Results

Squalene, labeled with tritium at C-9, -11, -14, and -16 and in the methyl groups attached at C-10 and C-15 (for simplicity in subsequent nomenclature these methyl carbons are numbered 10′ and 15′), was synthesized by exchange of the bis-Wittig reagent from 1,4-dibromobutane with tritiated *t*-butyl alcohol: *t*-butoxide followed by reaction with *trans*-geranyl acetone. Squalene-^{14}C was prepared from mevalonic-2-^{14}C acid by incubation with a rat liver homogenate. A mixture of squalene-^{14}C and squalene-^{3}H was then incubated with a rat liver homogenate to which cofactors had been added. The lanosterol was isolated and degraded as outlined in Scheme IV. Tables I, II, and III record the relevant activity data.

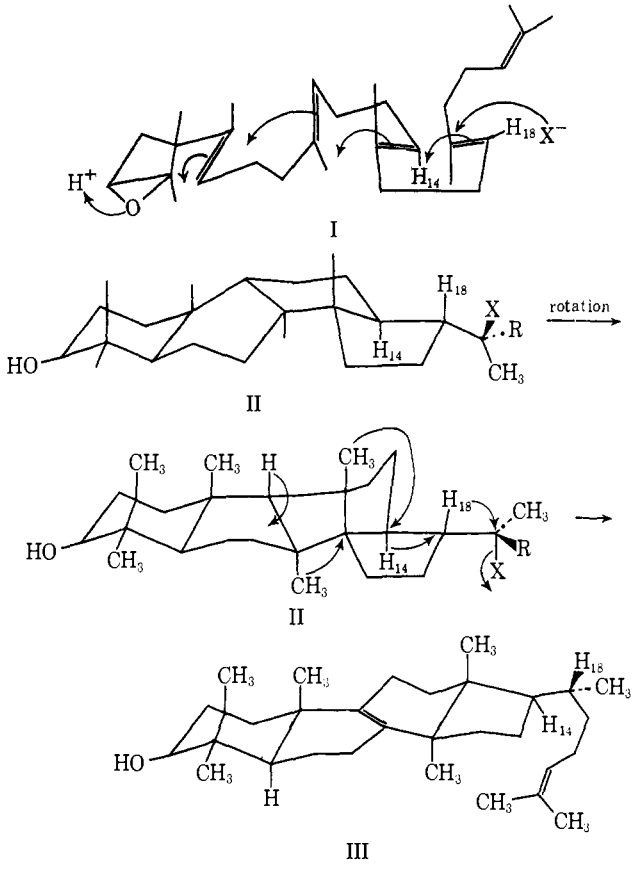
Table I. Conversion of Squalene-^{3}H,^{14}C to Lanosterol by Rat Liver Homogenate

	^{3}H/^{14}C ratio
Squalene (VI)	22.6 ± 0.7
Lanosterol (II)	19.6 ± 2.3

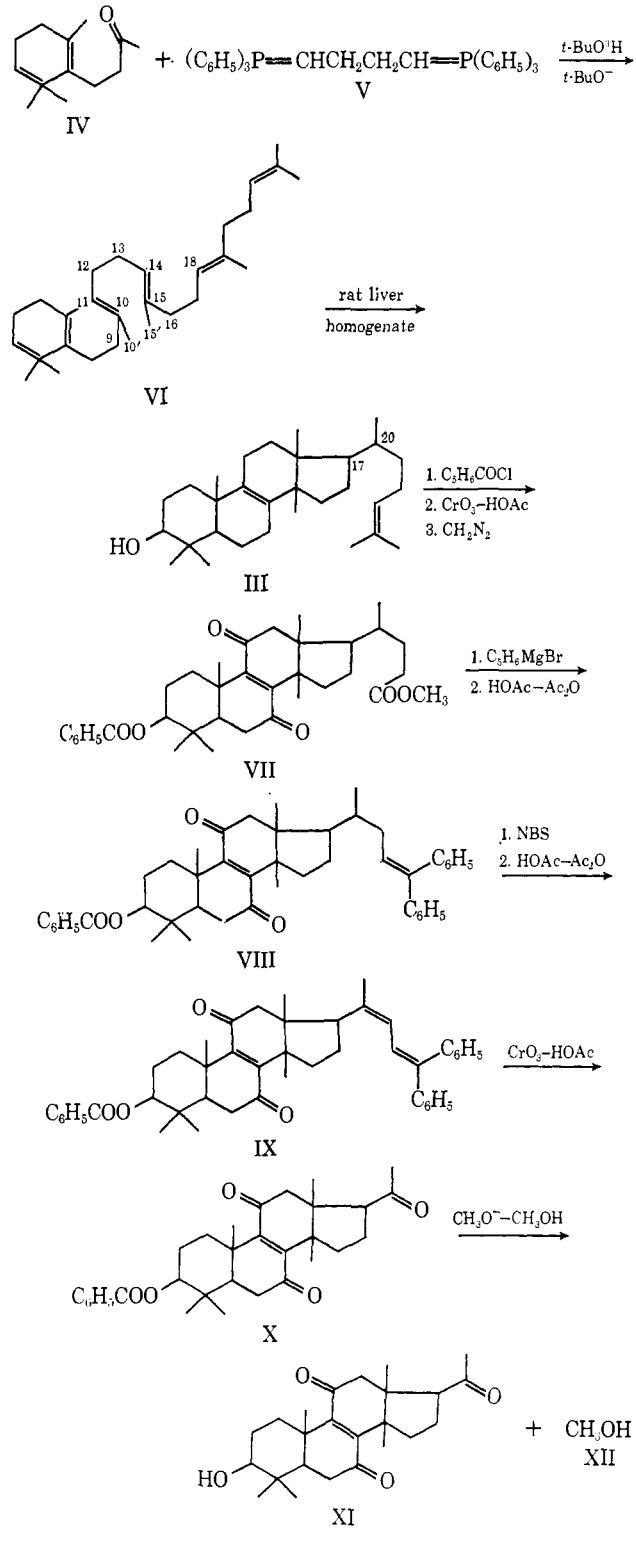
Discussion

The introduction of tritium into squalene by exchange of the bis-Wittig derivative of 1,4-dibromobutane de-

Scheme III



Scheme IV



pends upon the acidity of the protons adjacent to the phosphonium group. As the geranyl acetone is also exposed to the basic, tritiated *t*-butyl alcohol, tritium can be exchanged also into those positions which will become C-9 and C-16 and the angular methyl groups at C-10 and C-15 as well as into C-11 and C-14 of the resultant squalene. However, no tritium will be introduced at C-18 of squalene. There should, therefore, be no tritium at C-20 of the resultant lanostane skeleton, unless the hydrogen originally at C-14 of squalene should migrate to the C-20 carbon of lanosterol. The data in Table I show that about 13% of the tritium in squalene is lost during its conversion to lanosterol [(22.6 − 19.6)/22.6]100 = 13.3%. Because of the symmetry of the squalene molecule, the amount of tritium lost from C-11 during the biological conversion of squalene to lanosterol should be the same as that present in either C-17 or C-20 of the lanosterol.

The conversion of VII to IX should be attended with no loss of tritium activity if, in fact, none of the tritium at C-14 of the original squalene has migrated to C-20 of the lanostane skeleton. The data in Table II show that

Table II. Degradation of the Side Chain of Lanosterol

Compd	^{14}C activity, dpm/μmol	^{3}H activity, dpm/μmol	$^{3}H/^{14}C$ ratio
VIII	9.5	89.8	9.46 ± 0.27
IX	10.0	90.5	9.03 ± 0.23
X	7.7	90.7	11.60 ± 0.28
XI	7.7	79.0	10.25 ± 0.30

Table III. Exchange of X with Basic Methanol

Compd	Total ^{3}H activity in sample, dpm	^{3}H activity, dpm/μmol	^{14}C activity, dpm/μmol	$^{3}H/^{14}C$
X	1655	90.7	7.8	11.60
XI	1422	78.9	7.7	10.25
XII (recovered solvent)	178			

no significant amount of tritium is lost during this conversion. Thus, no appreciable amount of tritium, originally in C-14 of squalene, is present at C-20 of lanosterol.

The oxidative removal of C-22, -23, -24 of the original lanosterol in the conversion of IX to X should be attended with the loss of no tritium but of one of the five ^{14}C still remaining in the derivative IX. Mevalonic-2-^{14}C acid will yield lanosterol with ^{14}C at C-1, -7, -15, -22, -26, and -28. Oxidation of VI to VII will have removed one of the ^{14}C atoms; conversion of IX to X will thus result in loss of one-fifth of the remaining ^{14}C activity giving an expected ^{14}C activity in IX of $^{4}/_{5}(10.0) = 8.0$ and a $^{3}H/^{14}C$ ratio of $^{5}/_{4}(9.03) = 11.30$ in excellent agreement with the values of 7.7 and 11.60 determined experimentally.

The data in Table III demonstrate that equilibrium of X with sodium methoxide in methanol (with concomitant saponification of the benzoyloxy group at C-3) results in the loss of 13% of the ^{3}H present in X. (The methanol recovered after equilibration contains 11% of the total ^{3}H originally present in the sample of X that was equilibrated; therefore, the overall tritium balance is excellent.)

The anticipated loss of tritium from this equilibration should be $[13/(100 - 13)]100 = 15\%$ if all the hydrogen at C-17 of the sterol nucleus originated from C-14 of squalene and if the cyclization of squalene to lanosterol proceeds so that there is no appreciable effect of tritium *vs.* hydrogen at C-11 of squalene (C-9 of the sterol nucleus) on the overall rate of formation of lanosterol from squalene; that is, the loss of hydrogen from C-9 of a protosterol derivative occurs *after* the rate-determining state. The close agreement between the 15% tritium loss expected if these considerations obtain and the 13% determined experimentally thus strongly supports the conclusion that hydrogen at C-17 of the sterol nucleus originated at C-14 of the squalene precursor and that loss of the hydrogen from C-9 of the sterol nucleus occurs after the rate-determining step in the squalene–lanosterol conversion.

The hydrogen migrations are, therefore, a sequence of two 1,2-shifts. The hydrogen at C-17 of the protosterol cation moves to C-20 followed by migration of the hydrogen at C-13 of the protosterol cation (originally at C-14 of squalene) to C-17. These hydrogen migrations are followed in turn by sequential 1,2-shifts of the methyl groups at C-14 and C-8[16–18] and, finally, loss of a proton from C-9 completes the conversion of the protosterol structure to lanosterol. (This result, of course, does not formally exclude the proposal of van Tamelen,[6] which is, however, difficult to reconcile in a unifying way with such tetracyclic triterpines as damarenediol and the pentacyclic triterpenes; see ref 1, Chapter 10.)

Experimental Section

Materials and Methods. Scintillation Counting. Radioactivity in samples containing ^{14}C, ^{3}H, or both was determined by scintillation counting in a Packard Tri-Carb scintillation counter, Model 3324, for which channel settings and gain had been optimized. The efficiency of counting was monitored by using the automatic external standardization unit of this instrument.

Samples were counted in a toluene based solution which was prepared by diluting Packard 25X concentrated liquid scintillator (40 ml, Packard Instrument Co.) with reagent grade toluene to 1 l. [final solution contained 1,5-diphenyloxazole (PPO, 5 g/l.) and 1,4-bis(4-methyl-5-phenyloxazoyl)benzene (dimethyl POPOP, 0.1 g/l.)]. In all determinations a quantity of unlabeled substance equal to the amount of labeled substance subsequently counted was added to the standard ^{3}H and ^{14}C solutions from which the efficiencies of ^{3}H and ^{14}C were determined to correct for any quenching by the substance.

The chemicals used in these experiments and their sources are as follows: adenosine 5-triphosphate, from equine muscle, Sigma Chemical Co; glucose 6-phosphate disodium salt, Calbiochem; nicotine–adenine dinucleotide phosphate monosodium salt, Calbiochem; reduced nicotine–adenine dinucleotide disodium salt, Calbiochem; *dl*-mevalonic-2-^{14}C acid, dibenzyldiamine salt, Tracerlab.

Preparation of Rat Liver Homogenate.[18] Livers were excised from male Sprague-Dowley rats (130–165 g) shortly after they had been sacrificed by a blow on the head.[29] The livers were stored on ice until taken to a cold room (4°) where they were homogenized, usually within 30 min of sacrifice. The livers were forced through a stainless steel frit which contained about 50 holes of 1.5 mm diameter. This material was further homogenized in a Teflon and glass tissue homogenizer (0.006–0.009 in. clearance, Arthur H. Thomas Co.) in two volumes of pH 7.4 potassium phosphate buffer (0.03 *M* in nicotinamide, 0.004 *M* in magnesium chloride) per weight of fresh liver. Complete homogenization required three to five strokes. The homogenate was centrifuged at 12,000 g at 2°. The supernatant was filtered through cheese cloth. Yield about 15 ml of enzyme preparation S_{12} for 10 g of liver.

***trans*-Squalene-9,11,14,16,10′,15′-^{3}H. 1. Tetramethylene-1,4-bistriphenylphosphonium Bromide.**[30] To a 250-ml flask, equipped with reflux condenser and drying tube, was added 90 ml of acetonitrile (MCB reagent), triphenylphosphine (43.2 g, 165 mmol, MCB, mp 79–80°), and 1,4-dibromobutane (17.3 g, 80 mmol, MCB, redistilled, bp 63–65° at 5 min). The reaction mixture was heated under reflux for 2 days, after which the solids were dissolved in hot chloroform and precipitated by addition of ether. The product was dried under vacuum at 180° over phosphorus pentoxide for 2 days. The recovery was 57.7 g, mp 298–300° (lit.[30] mp 296–298°).

***t*-Butyl-^{3}H Alcohol.** To dry *t*-butyl alcohol (about 15 ml distilled from calcium hydride) was added tritiated water (about 500 mCi in about 0.1 ml; Nuclear, Chicago). After 2 hr, calcium hydride (2 g, 47.7 mmol) was added to the solution which was then heated under reflux for 2.5 hr. The *t*-butyl-1-^{3}H alcohol was then distilled (yield about 12 ml).

Squalene-9,11,14,16,10′,15′-^{3}H.[31] Potassium (320 mg-atom, 8.2 mmol) was added to 12 ml of the *t*-butyl-H^{3} alcohol from above. After 6 hr, the potassium had reacted completely and tetramethylene-1,4-bis-triphenylphosphonum dibromide (2.8 g, 3.8 mmol) was added to the solution. The flask was flushed with nitrogen and stoppered. After 16 hr, *trans*-granyl acetone (1.61 g, 8.4 mmol, 99+% *trans* by vpc analysis) was added to the reaction. After 5 hr the reaction was quenched by addition of water (15 ml). The aqueous phase was extracted with three 15-ml portions of ligroin (bp 30–60°). The ligroin phase was then washed with three 10-ml portions of water and three 10-ml portions of saturated sodium chloride solution and dried over sodium sulfate, yield 48.6 mCi.

The product was chromatographed over 60 g of Woelm activity II neutral alumina. Ligroin (bp 30–60°) removed squalene in the first four fractions which were combined and the ligroin removed yielding 1.5 g of crude squalene. This material was divided into four parts, each of which was applied to a thin layer chromatography plate (10 cm × 20 cm by 2 mm, Brinkman, silica gel F_{254}). The plates were developed in benzene–ligroin (1:1). The squalene bands (R_f 0.7) were scraped from the plates and diluted with ether, acetone, and methanol. Removal of solvents in a stream of nitrogen left about 600 mg of squalene.

To a flask containing the squalene was added 100 ml of methanol saturated with thiourea (1 g of powdered). After the solution had been stirred at 4° for 4 days, the solid thiourea clathrate was filtered from the solution and washed with three 5-ml portions of cold methanol.[32]

The thiourea clathrate was decomposed with water (75 ml) and the squalene extracted with three 20-ml portions of ligroin (bp 30–60°). The ligroin layer was washed with three 20-ml portions

(29) Homogenates prepared from older rats (400–500 g) were more active for synthesis of squalene but less active for the synthesis of sterols.

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of saturated sodium chloride solution and dried over sodium sulfate, yield 4 mCi.

Squalene-1,5,9,16,20,24-^{14}C.[33] Liver homogenate S_{12} (40 ml) was placed in a 250-ml erlenmayer flask. Nitrogen was passed through the homogenate until the oxygen content was less than 5% of that in a saturated water, as determined by a YSI oxygen monitor. For the duration of the reaction, a slow stream of nitrogen was passed through the solution. To this enzyme preparation was added *dl*-mevalonic-2-^{14}C acid (2–3 mg, 11.5–17 μCi, Tracerlab) as its N,N′-dibenzylethylenediamine salt. Each of the following cofactors was added initially and after 1 and 2 hr, adenosine triphosphate (59 mg, 0.1 mmol) reduced nicotine–adenine dinucleotide (7 mg, 0.01 mmol), nicotine–adenine dinucleotide phosphate (4 mg, 0.005 mmol), and glucose 6-phosphate (2 mg, 0.0067 mmol). After incubation for 3 hr on a shaker at 37°, the reaction was quenched by addition of 80 ml of 15% potassium hydroxide in 50% ethanol–water. The mixture was heated for 3 hr at 70°. After the hydrolysate was cooled to room temperature, it was extracted with three 50-ml portions of ligroin (bp 30–60°). The ligroin layer was washed with two 25-ml portions of water and three 25-ml portions of saturated sodium chloride solution and dried over anhydrous sodium sulfate. The ligroin was removed on a rotary evaporator, yield 3.4 μCi (about 50% from *d*-mevalonic acid). The total resulting product was chromatographed on a 2-g column of Woelm neutral alumina, activity II. The first fraction (ligroin 10 ml) contained squalene-^{14}C (1.3 μCi) and the second fraction (acetone–ether 1:1, 10 ml) contained sterols (2.1 μCi).

Preparation of Sterols from Labeled Squalene.[19] A sample of biosynthetic squalene-^{14}C (derived from *dl*-mevalonic-2-^{14}C acid) (25,000 dpm, about 1.2 mmol or about 0.5 μg) was added to a 25-ml test tube. Solvent was evaporated in a steam of nitrogen. To this lanosterol (100 μg), 1,2-propanediol (20 μl), acetone (50 μl), and rat liver homogenate (5 ml) were added. Each of the following cofactors was also added initially and after 2 hr: nicotine–adenine dinucleotide phosphate (2.2 mg, 0.0029 mmol) and glucose 6-phosphate (4.5 mg, 0.015 mmol). The reaction mixture was shaken at 37° for 3 hr, after which it was quenched by addition of 10 ml of 15% potassium hydroxide in 50% ethanol–water. This mixture was heated at 70° for 2 hr. After the hydrolysate had cooled to room temperature, it was extracted with three 5-ml portions of ligroin (bp 30–60°). The ligroin was washed with three 5-ml portions of saturated sodium chloride solution and dried over anhydrous sodium sulfate. The products were separated by thin layer chromatography on analytical plates (5 cm × 20 cm × 0.25 mm Brinkman, silica gel F_{254}) developed in 20% ether–benzene, yield lanosterol (6000 dpm, 24%), cholesterol (10,000 dpm, 40%). An identical procedure was used to convert squalene-9,11,14,16,-10′,15′-^{3}H to sterols.

Sterol Benzoate. The crude labeled lanosterol was benzoylated by reaction with benzoyl chloride in dry benzene–pyridine solution and the product crystallized from methylene chloride–methanol.

Methyl 25,26,27-Trisnor-3-benzoyloxy-7,11-diketolanost-8-en-24-oate.[34–36] To a solution of lanosteryl benzoate (409 mg, 0.77 mmol) in 7 ml of methylene chloride and 6.5 ml of acetic acid was added over 5 min a solution of chromium trioxide (832 mg, 8.23 mmol) in 8 ml of 90% acetic acid. The reaction was then heated at 4.5 hr and excess chromium trioxide destroyed with 10 ml of methanol. Evaporation of solvent yielded a dark oil which was dissolved in 30 ml of water and 30 ml of ether. After addition of sodium chloride and 1 ml of concentrated hydrochloric acid, the aqueous solution was extracted three times with ether. The ether solution was washed with water, and dried over anhydrous sodium sulfate. The ether was evaporated to yield a light yellow-green solid which was dissolved in 30 ml of ether. An ethereal solution of diazomethane (from 504 mg, 4.9 mmol of N-nitroso-N-methylurea) was added and the solution stirred at room temperature for 4.5 hr. Excess diazomethane was destroyed with acetic acid and the solvent evaporated.

The crude product was chromatographed on a silica gel column (36 g of silica gel Grace, mesh size 100–200; elution solvent—60 ml of benzene with a gradient to 10% ethyl acetate; fraction size about 12 ml). Fractions 23–25 contained 96 mg (52%) of a yellow substance which crystallized from absolute ethanol as yellow needles: mp 198–200°; (ir (KBr pellet) 1713, 1685, 1668, and 1270 cm^{-1}; no absorption in the region 1750–1730 cm^{-1}.

Anal. Calcd for $C_{37}H_{52}O_4$: C, 79.24; H, 9.35. Found: C, 79.56; H, 9.41.

Fractions 29–34 contained 125 mg (29%) of methyl 25,26,27-trisnor-3-benzoyloxy-7,11-diketolanost-8-en-24-oate which was recrystallized from absolute ethanol to give 84 mg (20%) of yellow needles, mp 195–200°.[37]

25,26,27-Trisnor-24,24-diphenyl-3-benzoyloxy-7,11-diketolanosta-8,23-diene. A solution of methyl 25,26,27-trisnor-3-benzoyloxy-7,11-diketolanost-8-en-24-oate (386 mg, 685 mmol) in 20 ml of dry benzene was slowly added (10 min) at room temperature to a solution of 1.6 ml of 3 *M* ethereal phenylmagnesium bromide (Arapahoe, 4.8 mmol) in 70 ml of dry benzene under a nitrogen atmosphere; a deep orange precipitate formed. The suspension was refluxed for 3 hr, cooled in an ice bath, and 80 ml of 1 *N* sulfuric acid was added. The aqueous phase was extracted with ether (two 50-ml portions and two 30-ml portions) and the combined ether solution washed with saturated sodium bicarbonate solution and dried over anhydrous sodium sulfate. The ether was removed to leave a yellow foam.

Without further purification, the crude carbinol was refluxed for 1 hr in 45 ml of acetic acid–acetic anhydride (10:1). The solution was cooled and 100 ml of ice water added. A precipitate formed which was extracted into ether (four 50-ml portions). The ether solution was washed with saturated sodium bicarbonate solution and dried over anhydrous sodium sulfate. The ether was evaporated to leave 670 mg of a brown oil.

The crude product was purified by chromatography on alumina (Woelm, activity grade I, 20 g in a column 7 cm long). Elution with 30 ml of benzene removed biphenyl. A further 80 ml of benzene and 100 ml of 1% ether in benzene removed the diphenyl olefin (170 mg, 37%) which gave, after recrystallization from *n*-butyl alcohol, 106 mg of yellow needles, mp 216–219°. Concentration of the mother liquor gave an additional 19 mg of product (total yield of recrystallized 25,26,27-trisnor-24,24-diphenyl-3-benzoyloxy-7,11-diketolanosta-8,23-diene is 27%) (lit.[34] mp 219°).

25,26,27-Trisnor-24,24-diphenyl-3-benzoyloxy-7,11-diketolanosta-8,21,23-triene.[36] To a solution of 25,26,27-trisnor-24,24-diphenyl-3-benzoyloxy-7,11-diketolanosta-8,23-diene (109 mg, 0.163 mmol) in 22 ml of carbon tetrachloride was added N-bromosuccinamide (43.6 mg, 0.245 mmol). The solution was irradiated with a 150-W bulb for 1 hr causing reflux. On cooling, succinamide precipitated and was filtered. The filtrate was evaporated and the residue dissolved in 9 ml of carbon tetrachloride and 1.5 ml of glacial acetic acid and 0.15 ml of acetic anhydride added. The solution was refluxed for 6 hr, the solvent evaporated, and the residue dried over potassium hydroxide in a desiccator. The resulting yellow solid was purified by chromatography on an alumina column (10 g of Woelm, activity grade I). Benzene (50 ml) did not elude any substance, but 200 ml of 4% ether in benzene removed 99 mg (91%) of product.

After recrystallization from 8 ml of acetone, 30 ml of methanol, 61 mg (56%) of bright yellow needles, mp 252–254°, are obtained. A further 12.3 mg (11%) could be recovered on concentrating the mother liquors.

22,23,24,25,26,27-Hexanor-3-benzoyloxy-7,11,20-triketolanost-8-ene. To a solution of 25,26,27-trisnor-24,24-diphenyl-3-benzoyloxy-7,11-diketolanosta-8,21,23-triene (65.6 mg, 0.98 mmol) in 5 ml of methylene chloride and 18 ml of glacial acetic acid was added a solution of chromic oxide (127 mg, 1.27 mmol) in 9 ml of 90% acetic acid and the mixture allowed to stand at room temperature for 4 hr. Excess chromic acid was destroyed with 10 ml of methanol and the solvent was evaporated leaving a black resin which was dissolved in 50 ml of ether and 10 ml of water. The aqueous phase was extracted with ether (five 30-ml portions), the combined ether extracts were washed with water and dried over anhydrous sodium sulfate. Evaporation of the ether left a colorless solid which was separated by preparative thin layer chromatography (E. Merck Darmstadt, tlc plates, silica gel F_{254}, 0.5 mm thickness, precoated, 20 × 20 cm). The plate was developed with 5% ether in benzene and two major bands were resolved, one of which consisted of benzophenone. The other band was extracted with acetone and, after evaporation, 46.5 mg (96%) of a slightly yellow substance remained which on recrystallization from 0.7 ml of *n*-butyl alcohol gave 36 mg (75%) of yellow needles, mp 221–223°. (A similar

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preparation of cold material yielded yellow needles, mp 221–223°.)

Anal. Calcd for $C_{31}H_{38}O_5$: C, 75.89; H, 7.81. Found: C, 75.78; H, 7.66.

Ir (KBr) showed 1669, 1680, 1708, 1280, and a shoulder at 1698 cm^{-1}; uv λ_{max} 272.5 mμ (log ε = 3.95).

Equilibration of 22,23,24,25,26,27-Hexanor-3-benzoyloxy-7,11,20-triketolanost-8-ene with Basic Methanol. A mixture of 22,23,24,25,26,27-hexanor-3-benzoyloxy-7,11,20-triketolanost-8-ene (9 mg, 0.018 mmol), 0.5 ml of 8% potassium hydroxide in methanol, and 0.5 ml of 50% aqueous methanol was refluxed for 4.5 hr, and the methanol then distilled. An additional 0.5 ml of methanol was added to the residue and distilled. The procedure was repeated with two 0.2-ml portions of methanol. The combined distillate, totaling about 1.8 ml, was counted in toluene to which an additional 4 ml of ethanol had been added to get a homogeneous solution.

To the residue of the distillation was added 8 ml of water and 0.1 ml of acetic acid. The mixture was extracted several times with a total of 25 ml of ether. After washing with a saturated solution of sodium bicarbonate and a saturated solution of sodium chloride, and drying over anhydrous sodium sulfate, the ether was distilled to leave a slightly brown residue which was purified by thin layer chromatography (E. Merck Darmstadt, tlc plates, silica gel F_{254}–0.5 mm of eluent 20% hexane–ether) to give 4.65 mg of a white solid, which was recrystallized from absolute ethanol and counted (uv λ_{max} 272 mμ (log ε = 3.93).

Stereochemistry of the Base-Induced Rearrangement of Epoxides to Allylic Alcohols

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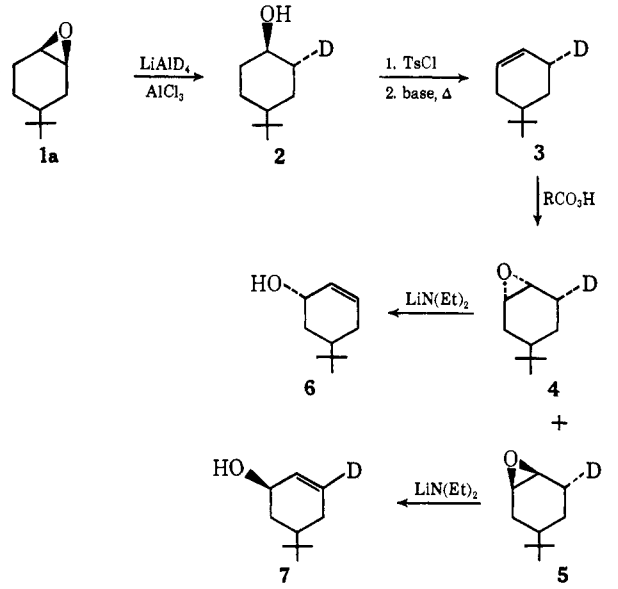
Abstract: The epoxide–allylic alcohol rearrangement of *cis*- and *trans*-4-*t*-butylcyclohexene oxide by lithium diethylamide is shown through appropriate deuterium substitution to occur by a *syn*-elimination process. Thus *cis*-3-deuterio-*trans*-5-*t*-butylcyclohexene oxide was converted to *trans*-5-*t*-butyl-2-cyclohexenol with loss of deuterium, while *trans*-3-deuterio-*cis*-5-*t*-butylcyclohexene oxide gave *cis*-5-*t*-butyl-2-cyclohexenol-3-*d* (loss of a proton).

The lithium diethylamide induced rearrangement of epoxides to allylic alcohols has two highly selective features which add greatly to its synthetic potential. Cope and Heeren[2] have shown that only *trans*-olefinic product is obtained from either *cis*- or *trans*-4-octene oxide. More recently, we have described[3] the very high selectivity of this base for proton abstraction from the least substituted β-carbon atom of an unsymmetrically substituted epoxide. These features in combination are unique to this elimination reaction, and consequently the mechanism is of considerable interest. This paper deals with a study of the stereochemistry of the lithium diethylamide induced rearrangement of substituted cyclohexene oxides.

Results

The determination of the stereochemical course (*i.e.*, whether *syn* or *anti*) of any elimination reaction requires that the relative configurations of the two asymmetric reaction sites and the geometry of the olefin product be known. The strong preference for proton abstraction from the least substituted center[3] adds to the difficulty of determining the preferred stereochemical course of the base-promoted epoxide rearrangement. Thus, under the usual reaction conditions (several hours in refluxing ether–hexane), no rearrangement of 2,5-dimethyl-3-hexene oxide (only tertiary β-protons available) is observed. Consequently it was necessary for the purposes of the present study to construct a model compound with a known H–D asymmetric secondary center. Scheme I outlines the major features of the synthesis used to obtain the model systems **4** and **5** and results obtained on rearrangement of these materials by lithium diethylamide. The precise details are somewhat more complicated than indicated here and require further discussion.



We have shown previously[3] that the reaction of lithium diethylamide with mixed *cis*- and *trans*-4-*t*-butylcyclohexene oxide (**1a, b**) is kinetically selective, such that the *trans* isomer **1b** is completely consumed in a time which allows only part of **1a** to react. This feature proved to be extremely useful in the present

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