SQUALENE CYCLIZATION

Thesis by
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To Mary Lou

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Finally, I would like to thank Eileen McKoy for her assistance in the preparation of this manuscript.

ABSTRACT

It has been observed that the proton, which is located at C-14 in squalene or C-13 in the "presterol cation," migrates less than 10% of the time (with a confidence level of 74%) to C-20 in lanosterol, and therefore 90% of the time (with a confidence level of 74%) to C-17 in lanosterol. If enzymatically controlled reactions are stereospecific, then at C-13/C-17/C-20 in the "presterol cation," there are two sequential 1, 2-migrations during the backbone rearrangement to form lanosterol. A theoretical interpretation of this observation, which reflects on the stereochemistry of the C-18/C-19 double bond of squalene just prior to cyclization, is presented.

A theoretical discussion of the minimal requirements for binding squalene-2, 3-oxide to an enzyme in order to obtain complete stereochemical control over the product is presented.

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Squalene Cyclization

I. INTRODUCTION

The complete structure of lanosterol was determined by Ruzicka and co-workers in 1952 (1). Shortly thereafter, Woodward and Bloch (2), and later Dauben (3), advanced a new proposal for the folding of squalene, which accounts for the labelling pattern of cholesterol produced from acetate. This new suggestion replaced an earlier one by Robinson (Figure 1, scheme a), which was not consistent with the structure of lanosterol, the newly proposed precursor of cholesterol. Ruzicka and co-workers (4, 5) have also advanced the "biogenetic isoprene rule" and have set forth a set of logically obtained rules for the cyclization of all trans-squalene. Included in this proposal is the second mechanism for the formation of lanosterol from squalene.

Observations from degradative studies on squalene (6) and cholesterol (7, 8, 9, 10) have proved consistent with the Woodward-Bloch proposal (Figure 1, scheme b).

In the cyclization mechanism set forth by Ruzicka (4, 5), the full steroid nucleus (all four rings) is formed synchronously and the resulting cation (see Figure 2) is internally stabilized by C-17 via sigma bond participation (Figure 2, step 1f). The result of the participation of C-17 is that the stereochemistry at C-19 is inverted. Subsequently, via a series of uninterrupted Wagner-Meerwein migrations, lanosterol is produced.

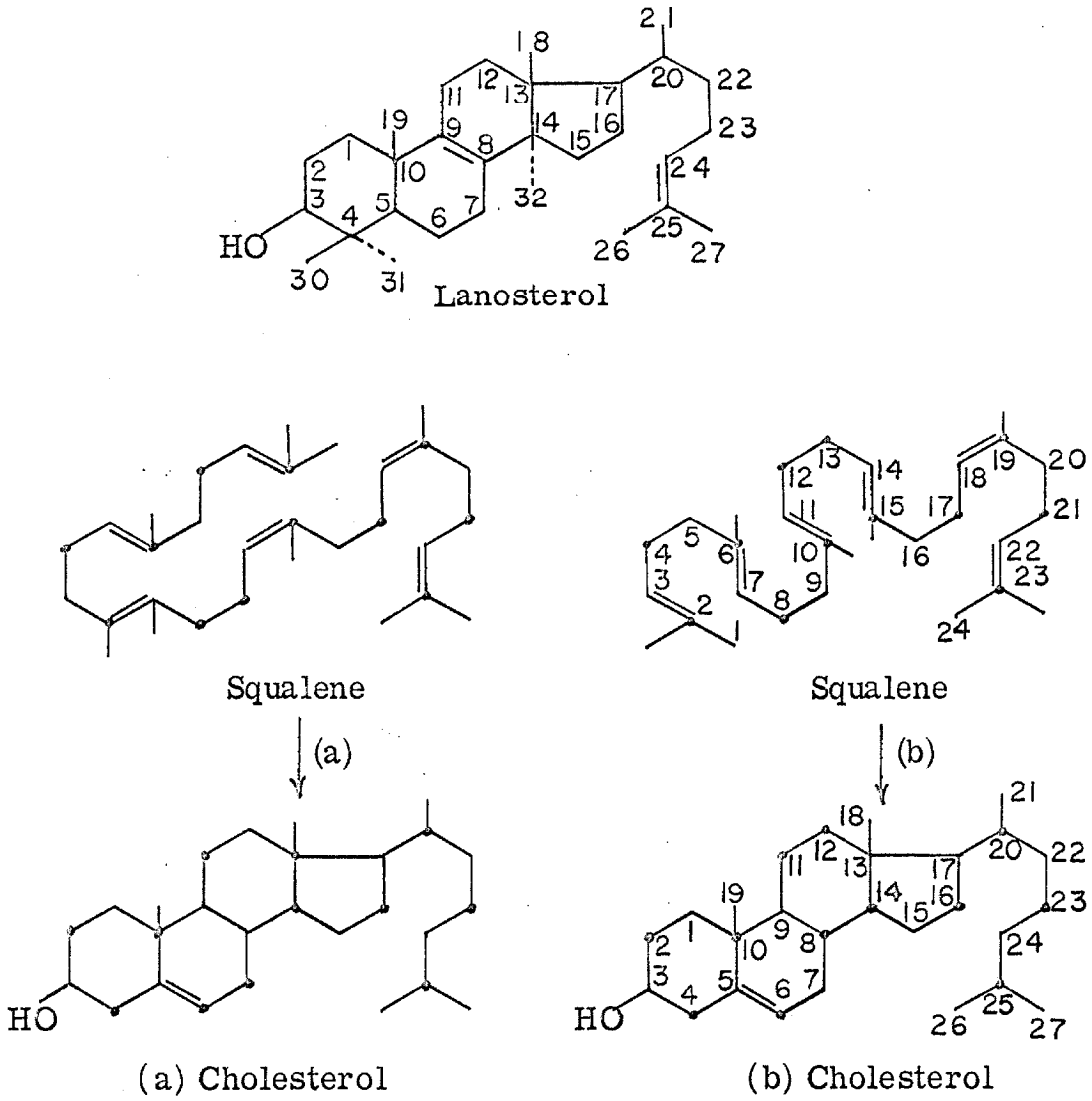
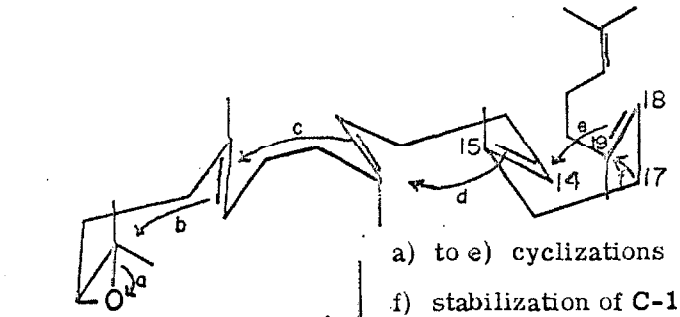


Figure 1. Proposed labelling patterns in cholesterol derived from carboxyl (•) labelled acetate via (a) Robinson's cyclization mechanism and (b) Woodward and Bloch's cyclization mechanism.

Ruzicka's Cyclization Mechanism

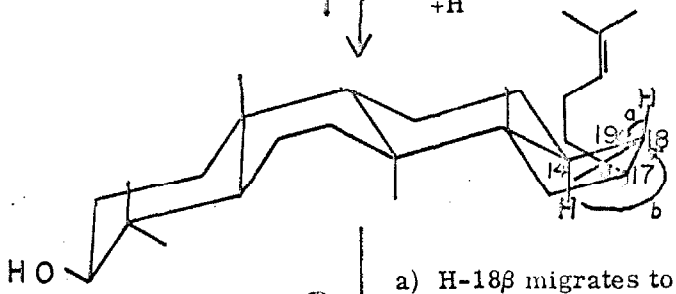


a) to e) cyclizations

f) stabilization of C-19 cation by C-17

$+H^+$

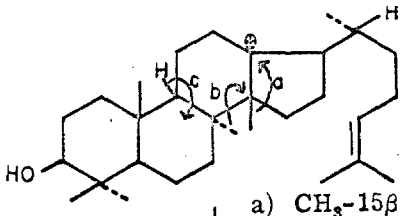
1



2

a) H-18β migrates to 19β

b) H-14α migrates to 18α

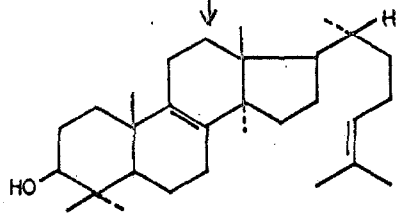


3

a) CH_3-15β migrates to 18β

b) CH_3-10α migrates to 15α

c) H-11β is eliminated



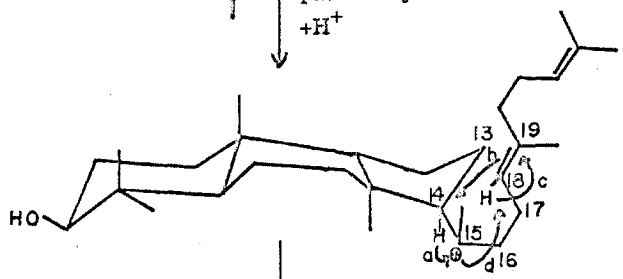
Lanosterol

Figure 2

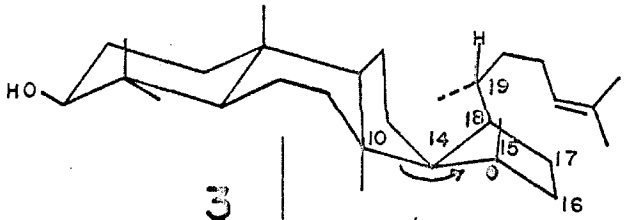
Van Tamelen's Cyclization Mechanism

Squalene-2, 3-oxide

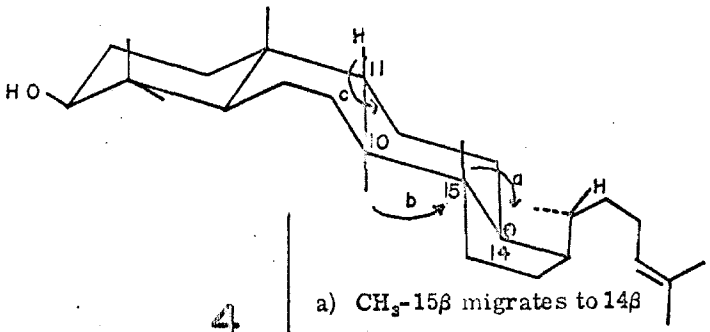
1 partial cyclization
$+H^+$



2
a) H-14α migrates to 15α
b) cyclization to spiro cation (charge at C-19)
c) H-18β migrates to 19β
d) H-15α migrates to 18α



3
C-10/C-14 bond migrates to C-10/C-15 position



4
a) CH_3-15β migrates to 14β
b) CH_3-10α migrates to 15α
c) H-11β is eliminated

Lanosterol

Figure 3

In step 1d (see Figure 2) of the cyclization, the cyclization is directed so that a secondary cation is produced at C-14, while an alternate cyclization could have led to a tertiary cation at C-15, which might be thought to be more stable and therefore favored.

In order to avoid this possibly less favorable secondary cation, van Tamelen, who has recently discovered an intermediate between squalene and lanosterol, i.e., squalene-2,3-oxide (11), has proposed a third cyclization mechanism (Figure 3) (12). This mechanism diverges from that of Ruzicka at step 1d, where it proposes the formation of a tertiary cation intermediate, which, after rearrangement to another tertiary cation (Figure 3, step 2a) cyclizes to a spiro cation. Thereafter, two proton migrations and expansion of the third ring bring this mechanism to a point where it is again identical with the one proposed by Ruzicka.

It should be noted that although van Tamelen's proposal alleviates the potential problem of forming a secondary cation instead of a 3° cation, it is no longer a nonstop sequence. Specifically, in step 2a, the C—H bond at C-14 is orthogonal to the developing positive p orbital at C-15. Therefore, the migration of H-14 must be preceded by rotation of the C-14/C-15 bond or addition of a nucleophile at C-15, followed by rotation of the C-14/C-15 bond and displacement of the nucleophile during step 2a. Additionally, step 3 suffers from a similar difficulty. In the previous step (2d), a proton migrated from the α position of C-15, so that if the methyl group attached to C-15 is to remain in the required β position, the migration of the C-10/C-14

(7) This cyclase requires no cofactors and no oxygen (18).

(8) All trans-squalene is the biologically active isomer (19).

The above observations place certain limits on the mechanism of converting squalene to lanosterol.

(1) Except for the exchangeable hydroxyl position, if any proton from water is added to either squalene or 2, 3-oxidosqualene, it must be subsequently eliminated before the presently recognized stable products, 2, 3-oxidosqualene and lanosterol, are formed.

Thus, the following intermediates (I, II) would be allowed (12).

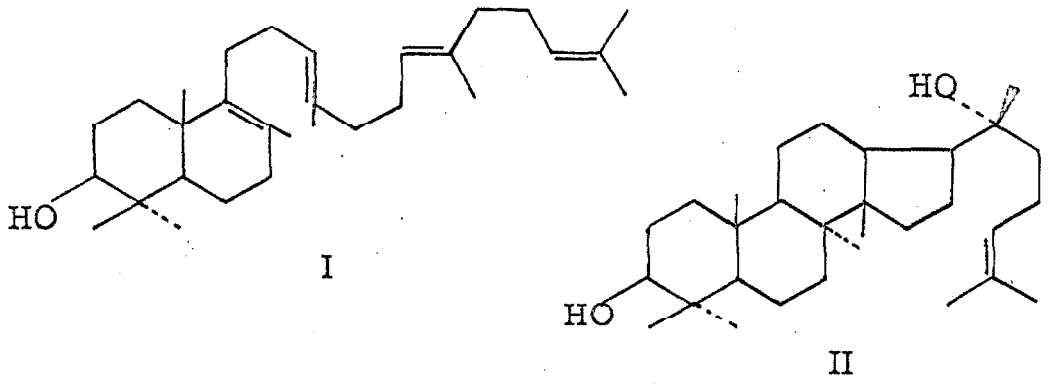


Figure 2

(2) Although all trans-squalene is the starting form of the C_{30} chain, a cis-trans isomerization, as by addition of a nucleophile (12), possibly at the cation stage, followed

by a rotation and re-elimination of the nucleophile, can not be ruled out.

(3) Addition of a nucleophile, e.g., a part of the enzyme or water, to any double bond except C-22/C-23 is consistent with the above data.

This work will show that most probably the sequence of the hydride migrations in the "presterol" cation is from C-13 to C-17 and C-17 to C-20 and that on theoretical grounds, the trans geometry in the pre C-17/C-20 double bond is required by this sequence of migrations.

II. DISCUSSION

A. Proton migrations

It is known that when squalene produced biosynthetically from mevalonic-4R-4-^{3}H acid, is enzymatically cyclized to lanosterol and cholesterol, one tritium is eliminated, one tritium is found in sterols at the 17-α position, one at either C-20 or C-22, one in the fragment C-23 to C-27, one at position 3-α, and one most probably at position 5-α (20).

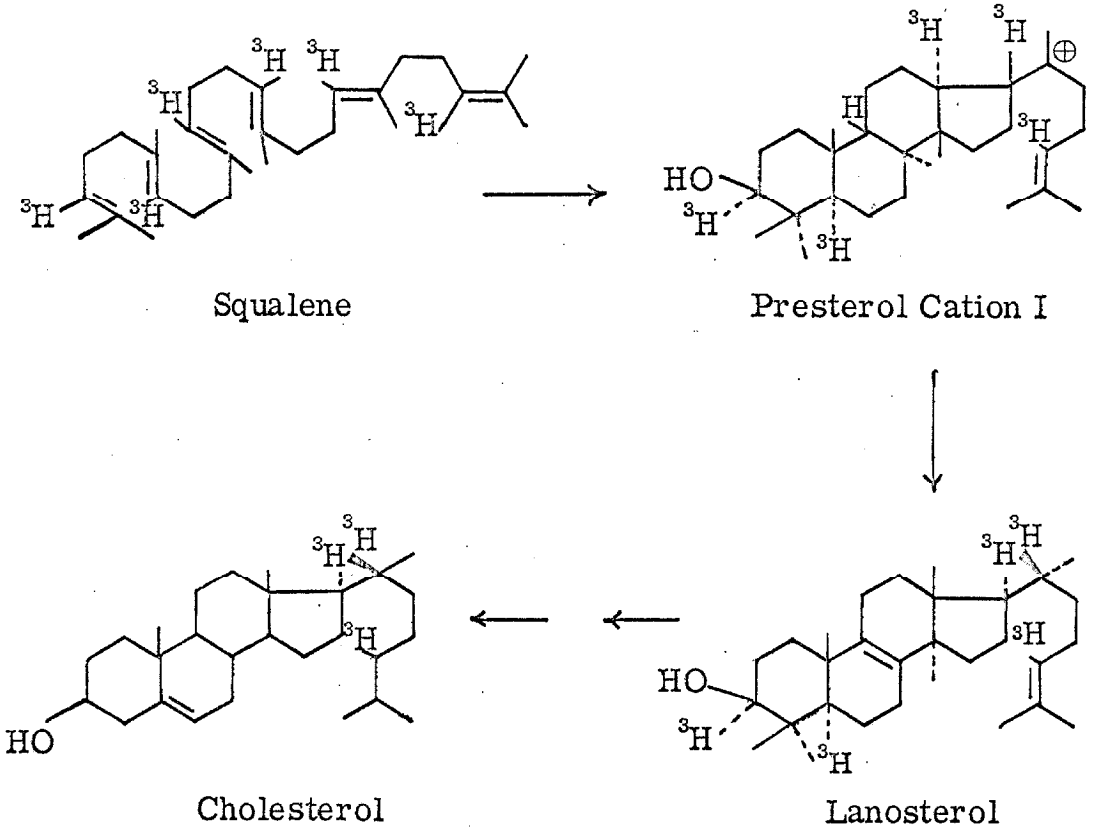


Figure 5

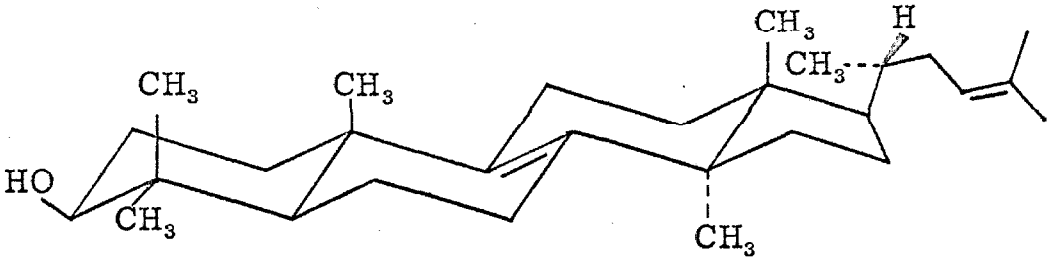
In figure 5 , the "presumed" actual locations of all the tritium labels are shown.

As can be seen, a label appears at position 17-α in both the "presterol cation" (I) and subsequent products. Therefore, it has not been determined whether that tritium, which is at position 17-α in cholesterol, is the same one that was at position 17-α in the "presterol cation."

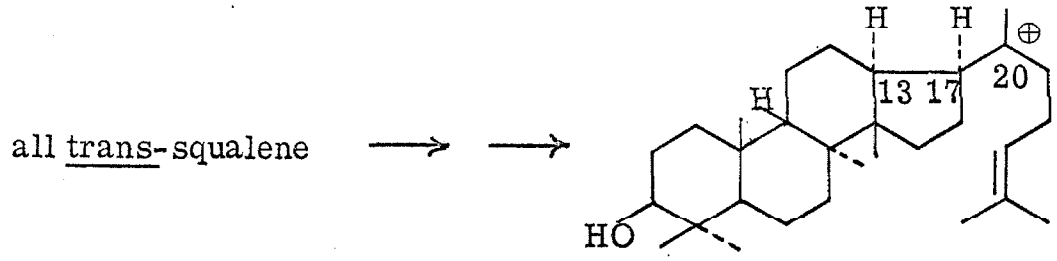
Although it has been shown that natural squalene has the all trans form (19), isomerization just prior to cyclization, as by a nucleophilic attack and elimination, perhaps during the cyclization, has not been eliminated. However, since the stereochemistry of the product lanosterol is known, the stereochemistry of squalene, just prior to cyclization, can be inferred if the migration sequence at C-13/C-17/C-20 is known.

For example consider the outcome of the following migration sequence on all trans-squalene. If the migration sequence is from C-13 to C-20 (i.e., 1, 3-migration), it is a reasonable assumption that the stereochemistry at C-17 would remain unchanged. Therefore the proton at C-17 is in the α position in the "presterol cation," as it is found in lanosterol. If the methyl migrations are of the Wagner-Meerwein type, whereby the stereochemistry at each carbon atom is inverted during each migration, then the proton at C-13 must be in the α position, since C-18 methyl is ultimately in the β-position. Furthermore, if it is assumed that the positive charge generated at

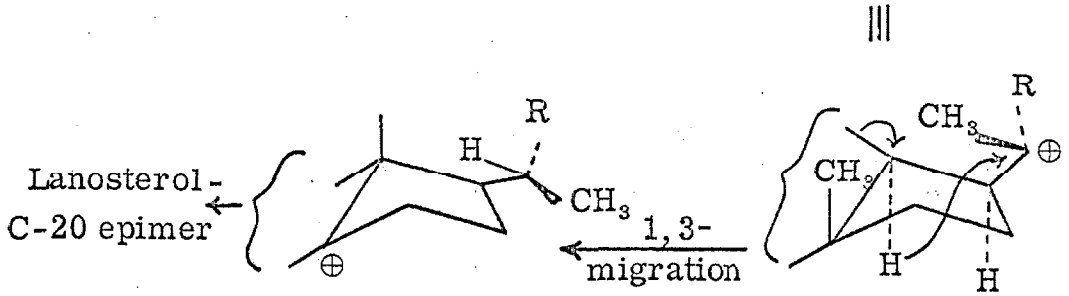
C-20 is stabilized from the side distal to the C-13/C-17 bond, then migration of the proton at C-13 to C-20 with inversion at C-20 results in a product which is the C-20 epimer of lanosterol.



Lanosterol



Presterol Cation II



$R = C_6H_{11}$

Figure 6

If the same C-13 to C-20 migration sequence is considered for squalene which has one cis double bond at C-18/C-19, the result is that the product has the same stereochemistry as lanosterol. Therefore, if a 1, 3-migration pattern were observed in the rearrangement of the "presterol cation," it could be inferred that, just prior to cyclization, the C-18/C-19 bond in squalene was cis.

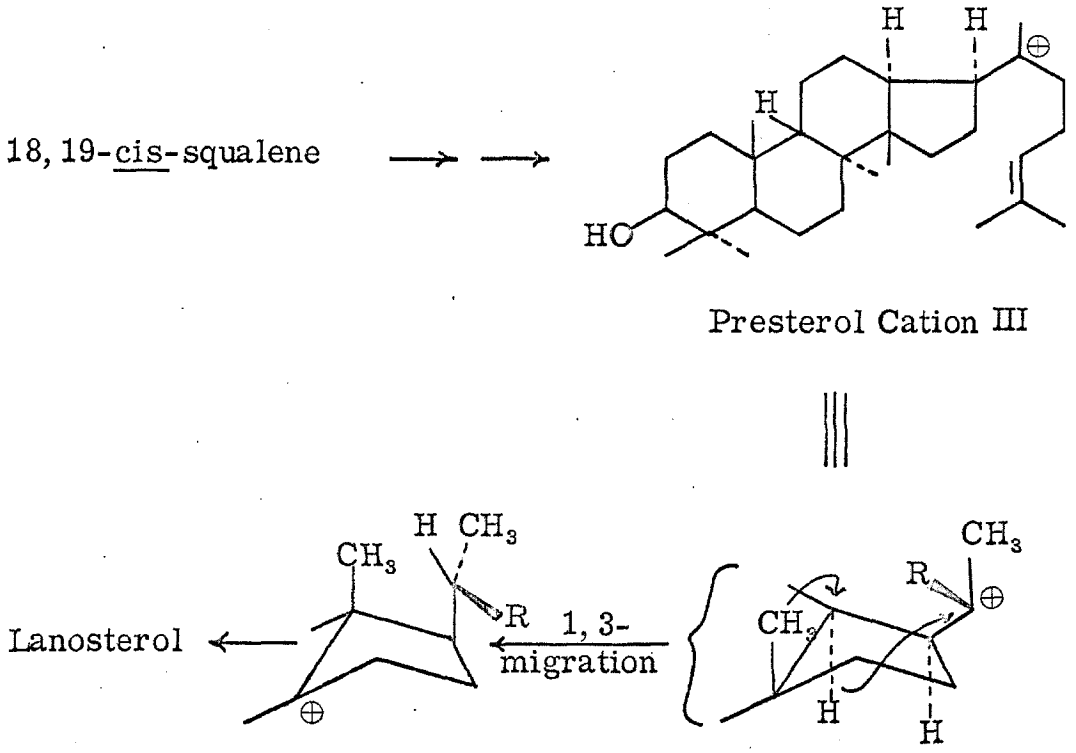


Figure 7

On the other hand, if two sequential 1, 2-migrations are considered (i.e., proton from C-17 to C-20 and proton from C-13 to C-17), the outcomes are reversed. Now, the stereochemistry at C-17 must be inverted and therefore the proton at C-17 must be in the β

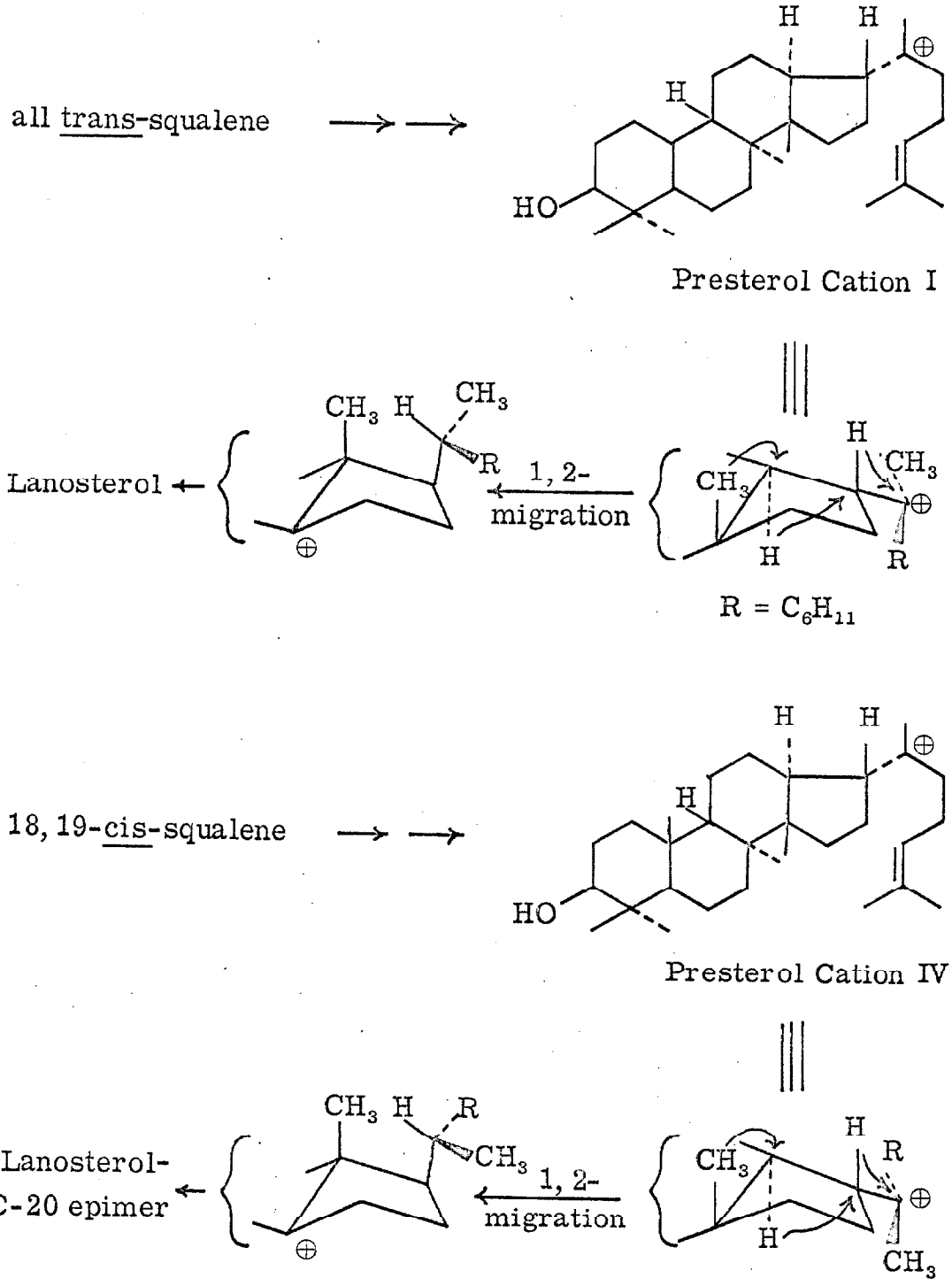

all trans-squalene
Presterol Cation I
Lanosterol
1, 2-
migration
R = C6H11
18, 19-cis-squalene
Presterol Cation IV
Lanosterol-
C-20 epimer
1, 2-
migration

Figure 8

position in this presterol cation. In the case of all trans-squalene, if the same assumptions about the stabilization of the positive charge at C-20 are invoked, migration of the proton from C-17 to C-20 and continued Wagner-Meerwein migrations yield lanosterol as the product. In contrast, such sequential 1, 2-migrations on the "presterol cation" formed from 18, 19-cis-squalene results in a structure identical to lanosterol, except for the sterochemistry at C-20.

It should be explained that the stabilization of the positive charge at C-20 is not unusual or new (21). Such stabilization is required in the rules for cyclization of all trans-squalene, which were set forth by Ruzicka (4, 5). In his case the stabilization was specifically internal, i.e., by carbon-carbon sigma bond participation. However, this more general treatment includes internal stabilization as well as possible stabilization by the enzyme involved in the cyclization.

The results of an experimental test of this migration sequence will be given in section III.

This discussion has reflected mainly on the stereochemistry of the D ring of triterpenes. Some of the stereochemistry of this carbocyclic system is certainly controlled by the enzyme responsible for squalene cyclization. However, it seems likely that the enzyme need not "hold rigidly" the whole squalene molecule in order to completely control the stereochemistry of the whole carbocycle which is formed.

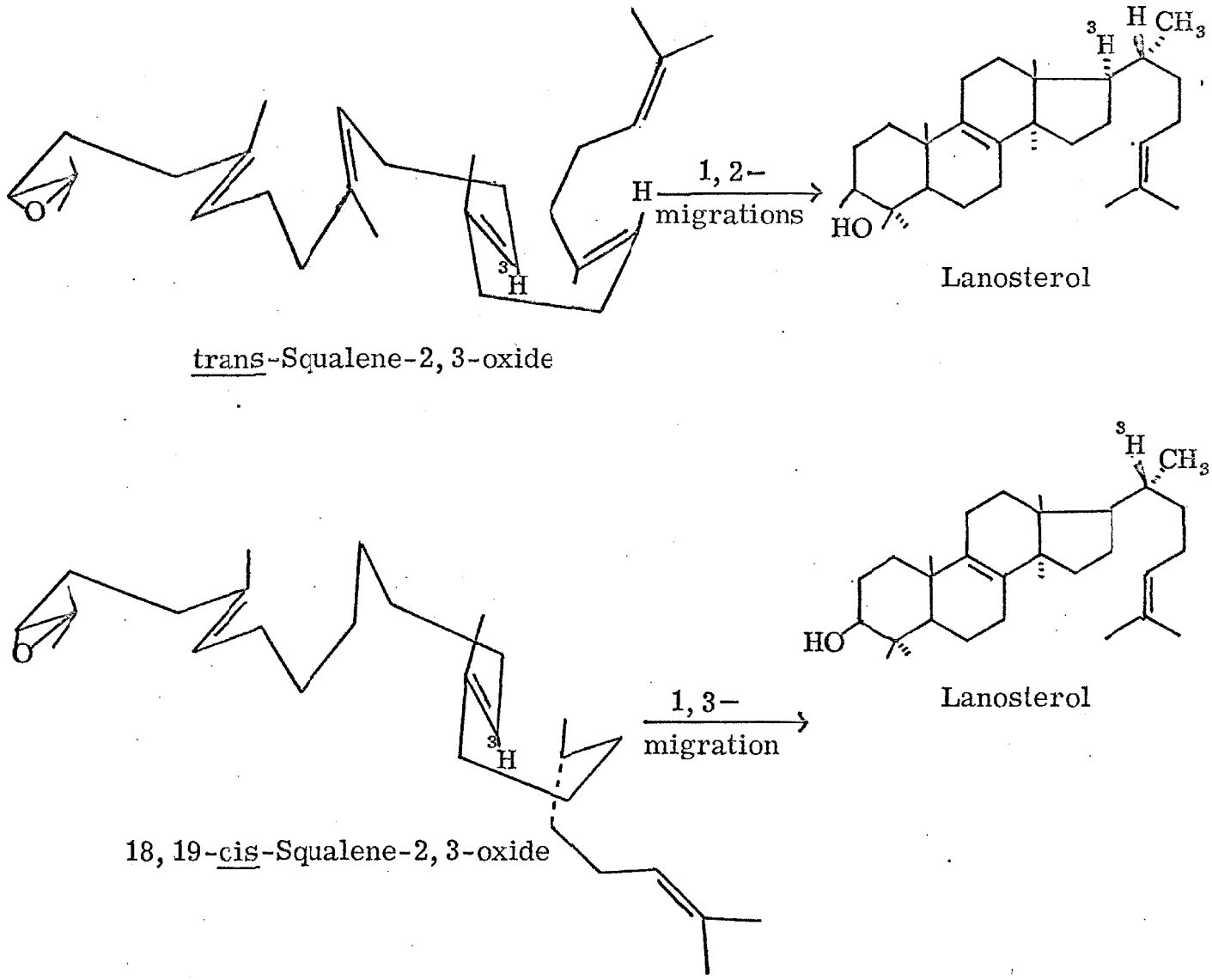

H
1, 2–
migrations
HO
Lanosterol
trans-Squalene-2, 3-oxide
1, 3–
migration
HO
Lanosterol
18, 19-cis-Squalene-2, 3-oxide

Figure 9

B. Chain folding

In the mechanisms for the cyclization of squalene proposed by Ruzicka (4) and van Tamelen (12), the basis of their arguments is analogy to known chemistry. Thus, one of the requirements set forth by Ruzicka (5) is that sequentially migrating groups be trans to each other and coplanar. The mechanism of van Tamelen (12) was proposed to avoid invoking the formation of a secondary cation in preference to a tertiary cation.

It is certainly necessary to consider known chemistry when proposing a biosynthetic mechanism, but it would also seem to be useful to consider the biological implications of such proposals.

One of the functions of squalene cyclase is to "hold the squalene chain in the conformation necessary for forming the final product." Possibly it was considered that this meant that all parts of the chain which eventually became part of the carbocyclic structure were held in the "proper orientation."

For the tetracyclic products of squalene, the minimal binding requirements, a biologically desirable consideration, to exert full stereochemical control of the products have been considered. The results of this consideration will be offered here.

Initially three assumptions must be made: (1) that sequentially migrating groups must be trans to each other and coplanar [one of Ruzicka's rules (5)], (2) that there is a significant energy release by forming a five- or six-membered carbocyclic ring when a double bond is broken (2 carbon-carbon single bonds, 80 kcal each—

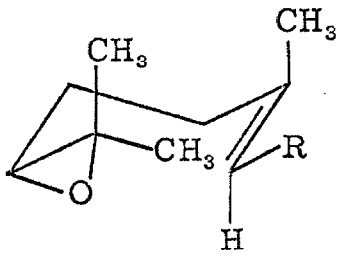
1 carbon-carbon double bond, 142 kcal; net gain 18 kcal), and (3) that the migrations along the backbone are energetically favorable, e.g., for strain relief (22).

Since the epoxide ring in squalene-2, 3-oxide is opened during cyclization, it seems reasonable to assume that this is one of the likely sites for binding to an enzyme. If then the incipient A ring is held in a "chair" conformation (as much a chair as can be made from a cyclohexadiene type structure), then the 19-methyl group in a resulting tetracyclic compound would be in the β position, as it is always found. Therefore it will be assumed that the A ring is held in this "chair" conformation.

There are two possibilities for the B ring, either incipient chair or incipient boat. First consider the incipient chair structure. Here the C-8 methyl of the cation would be held in the β position and the C-9 proton in the α position, so that if there is to be any backbone migration, these two substituents are in the required trans coplanar relation.

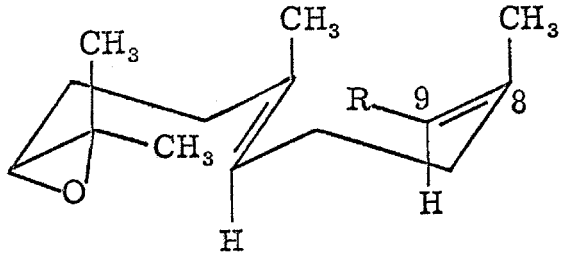
At this point, complete steric control over the rest of the cyclization is imposed, for there is only one conformation of ring C which places the C-14 methyl group of the cation trans and coplanar to the C-8 methyl group of the cation. That conformation is incipient chair, which restricts the C-14 methyl group to the α position and the C-13 proton to the β position.

Ring A (chair)



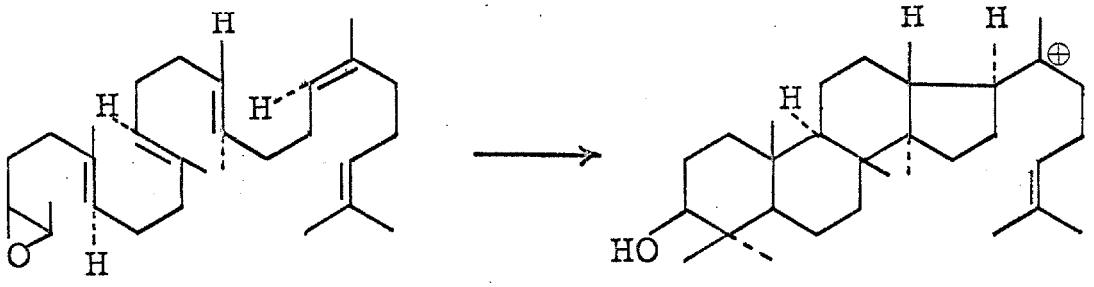
$R = C_{21}H_{35}$

Rings A and B (chair-chair)



$R = C_{16}H_{27}$

Squalene-2, 3-oxide



Squalene-2, 3-oxide
(chair-chair-chair-boat)

Chair-Chair-Chair-Boat
Cation (C-C-C-B)

Figure 10

Assume that there will be 1, 2-migrations at C-13/C-17/C-20. Then there is only one conformation for the D ring, which is the incipient boat conformation. Thus the C-17 proton of the cation is placed in the α position.

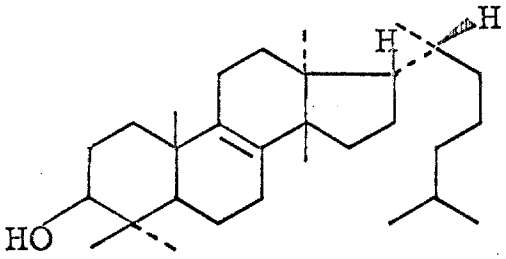
A number of tetracyclic triterpenes have structures which indicate they could have arisen from such a cation (23). Indeed, the variety is so great that the possibility of a full positive charge being located at C-20 seems highly likely.

If both the structures of dammaradienol, dammarenediol I and dammarenediol II (Figure 11) and the fact that these compounds are obtained from the same source (22) are considered, it would seem possible that they all arose from the same cation (Figures 10, 11, C-C-C-B) via elimination or hydroxyl capture.

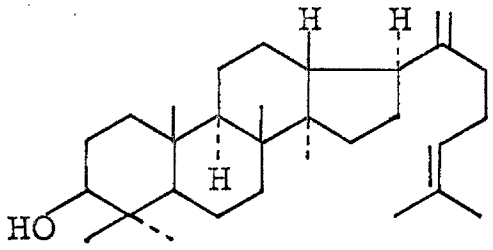
The other possibility for ring B is that it is held in an incipient boat. In this case, the C-8 methyl would be held in the α position and the C-9 proton in the β position. This requirement forces ring C to be in the incipient boat form, where the C-14 methyl assumes the β position.

Again assume that there will be 1, 2-migrations at C-13/C-17/C-20. Since the C-13 proton is held in the α position, the C-17 proton must be held in the β position, which forces ring D into the incipient boat form.

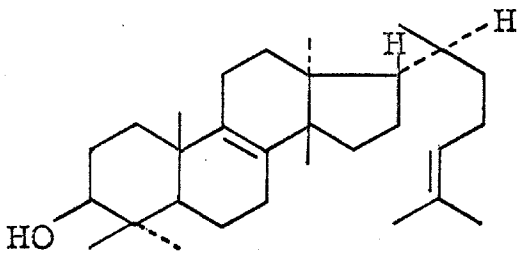
Unfortunately, there are few triterpenes of this structure. Lanosterol and parkeol are the only good examples (Figure 12).



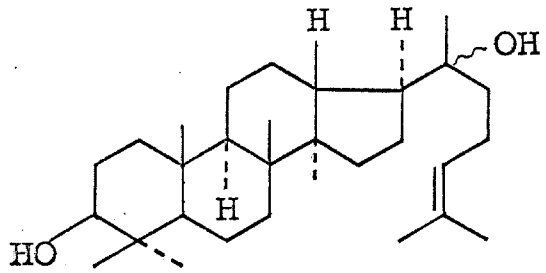
Euphol



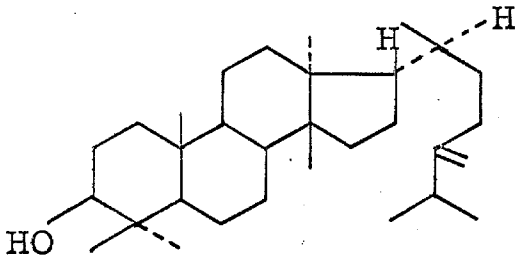
Dammaradienol



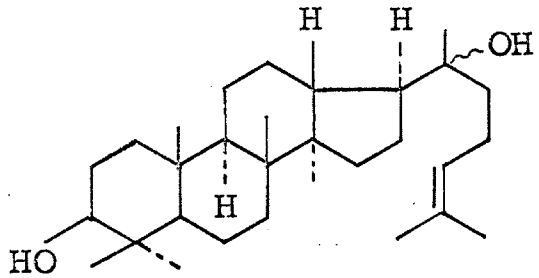
Tirucallol



Dammarenediol I (20 ξ_1)



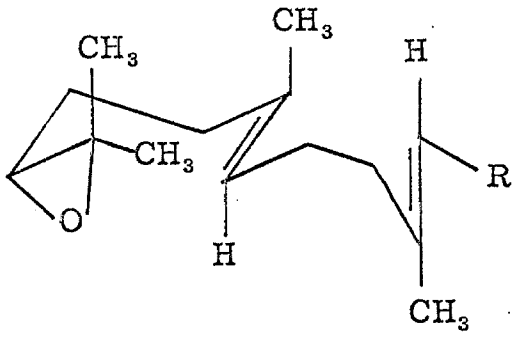
Euphorbol



Dammarenediol II (20 ξ_2)

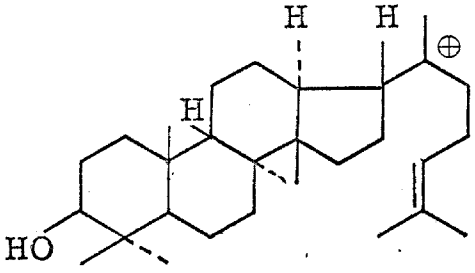
Figure 11

Rings A and B (chair-boat)

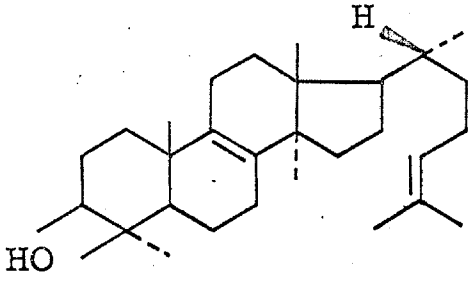


$R = C_{16}H_{27}$

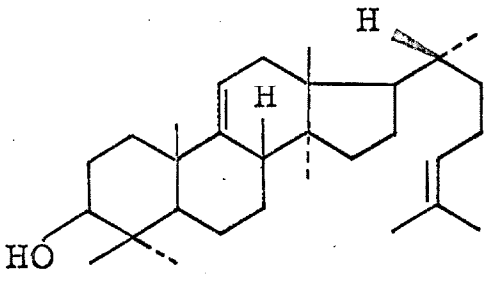
Squalene-2, 3-oxide



Chair-Boat-Chair-Boat Cation (C-B-C-B)



Lanosterol



Parkeol

Figure 12

As will be remembered from the prior discussion on migrations, the stereochemistry of C-17 and C-20 are equally dependent on the "folding of the molecule" and the migration sequence at C-13/C-17/C-20. Whether the migration sequence is 1, 2 or 1, 3, the folding can be changed to accommodate either mechanism.

Since cyclization is thermodynamically favored by 18 kcal per ring formed, and backbone rearrangement is probably stabilizing (e.g., strain relief), then once the A and B rings are formed, the greatest energy gain can only be realized in a single folding arrangement. Therefore, the minimal requirements for binding squalene-2, 3-oxide to an enzyme need include only holding rings A and B in the proper stereochemical relation to obtain a fully cyclized product (or products) containing only one stereochemical structure.

III. RESULTS

All trans-squalene-11,14-$^{3}H_2$ was synthesized by a previously reported method (24, 25) (Figure 13). Some tritium also exchanged into the methyl groups attached to C-10 and C-15, but this was not detrimental to the experiment.

In Experiment A (see p. 44) this specifically labelled squalene was combined with squalene labelled with ^{14}C at positions 1, 5, 9, 16, 20 and 24, which had been prepared enzymatically from mevalonic-2-^{14}C acid. This doubly labelled squalene was converted to sterols with an enzymatically active homogenate of rat liver. The sterols were separated by thin layer chromatography and lanosterol was extracted. This material was recrystallized, along with added lanosterol, until the $^{3}H/^{14}C$ ratio remained constant and the $^{3}H/^{14}C$ ratio of the material in the supernatant from the crystallization was identical to the ratio of the lanosterol from which it was filtered.

This material was converted to lanosteryl benzoate, which was purified by thin layer chromatography. The lanosteryl benzoate was oxidized with chromium trioxide and the resulting acid was methylated with diazomethane. This material was purified by crystallization from ethanol and by thin layer chromatography. At this point only a small number of ^{14}C counts remained, so a number about equal to those already present was added as compound III (this material was obtained from a previous experiment). Compound III was reduced with zinc and acetic acid to give compound IV.

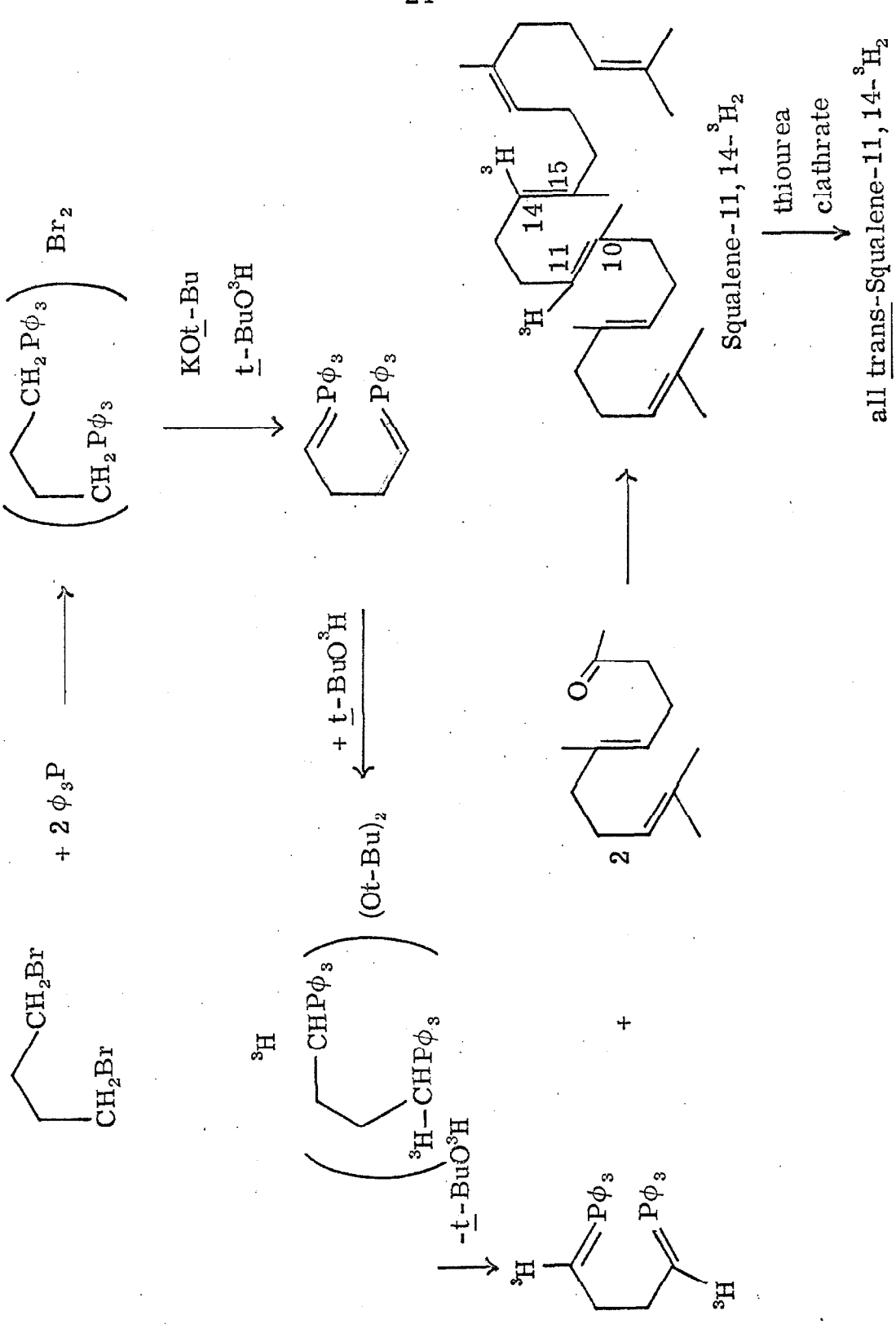

CH2Br
CH2Br
+ 2 φ3P
CH2 Pφ3
CH2 Pφ3
Br2
KOt-Bu
t-BuO3H
Pφ3
Pφ3
+ t-BuO3H
(Ot-Bu)2
3H
CHPφ3
3H–CHPφ3
-t-BuO3H
3H
Pφ3
Pφ3
3H
+
O
2
3H
14
15
11
10
3H
Squalene-11, 14-3H2
thiourea
clathrate
all trans-Squalene-11, 14-3H2

Figure 13

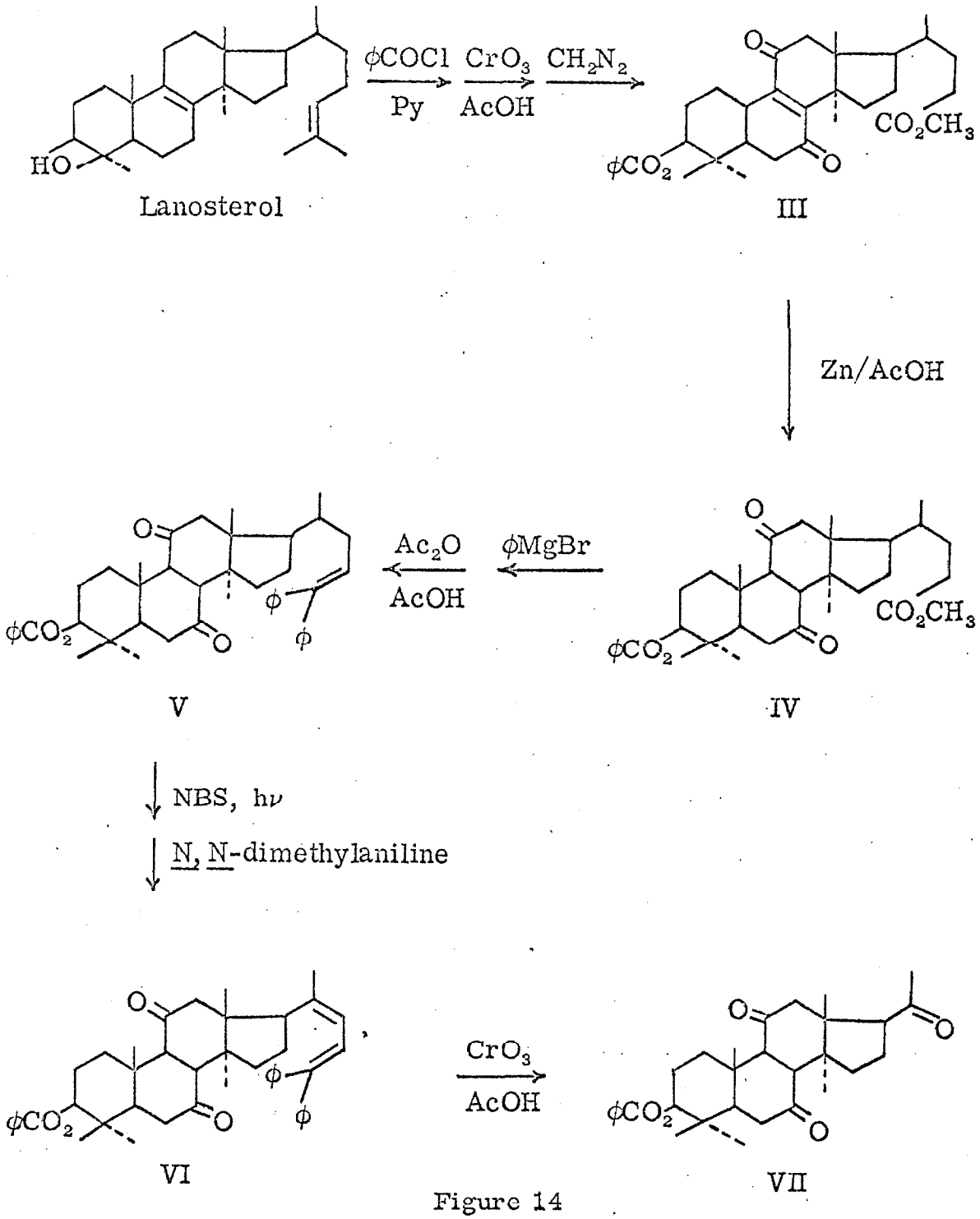

φCOCl
Py
CrO3
AcOH
CH2N2
HO
CO2CH3
φCO2
O
Lanosterol
III
Zn/AcOH
Ac2O
AcOH
φMgBr
φ
IV
V
NBS, hν
N, N-dimethylaniline
VI
VII

Figure 14

Table 1	$^{3}H/^{14}C \pm 1\sigma$
Squalene	22.6 ± 0.7
Lanosterol	16.8 ± 1.3; 19.6 ± 2.3
Compound III	24.0 ± 2.2
Compound III + ^{14}C	12.0 ± 2.2
Compound IV	14.2 ± 0.8

These data can be interpreted as showing that the amount of tritium at C-11, and therefore at C-14, in this sample of squalene-11, 14-$^{3}H_2$ is between 25.8 ± 6.5% and 13.3% ± 10.6% of the total amount of tritium in the sample.

$$\begin{array}{r} 22.6 \pm 0.7 \\ -16.8 \pm 1.3 \\ \hline 5.8 \pm 1.5 \end{array} \qquad \frac{5.8}{22.6} = 25.8, \qquad \frac{1.5}{5.8} \times 25.8 = 6.5$$

$$\begin{array}{r} 22.6 \pm 0.7 \\ -19.6 \pm 2.3 \\ \hline 3.0 \pm 2.4 \end{array} \qquad \frac{3.0}{22.6} = 13.3, \qquad \frac{2.4}{3.0} \times 13.3 = 10.6$$

Consequently, in lanosterol produced from this squalene, of the total amount of tritium in the sample between 25.8/(100-25.8) = 34.8% ± 9.2% and 13.3/(100-13.3) = 15.4% ± 13.6% should be at C-17 or C-20.

Much greater confidence is placed in the higher values (25.8% and 34.8%) since these result from counting a sample of lanosterol after it had been purified by thin layer chromatography and crystallized

from ethanol. The other values (13.3% and 15.4% were obtained after the second recrystallization of lanosterol. Since the $^{3}H/^{14}C$ ratio (16.8 ± 4.6) for the material in the supernatant from the second recrystallization is almost the same as that for the crystalline lanosterol (and it should be much lower if the $^{3}H/^{14}C$ ratio of the crystalline product were to increase) and the specific activities of the crystalline material and the material from the supernatant are also the same (see p. 44), there is good reason to believe that the material obtained after the first crystallization was homogenous and that the $^{3}H/^{14}C$ ratio associated with this material was correct. Indeed, the large error in the $^{3}H/^{14}C$ ratio from the twice crystallized material indicates that it could, in fact, be the same ratio as that from the singly crystallized material, which ratio was obtained with greater accuracy.

Moreover, when squalene-11,14-$^{2}H_2$ was synthesized by the same method (25), the amount of deuterium at C-11 and C-14, as determined by pmr and mass spectroscopy, was approximately 83% of the total deuterium incorporated in the whole molecule. Therefore, it seems reasonable to assume that a large amount of the tritium incorporated should have been at C-11 and C-14 (51.6%, if the higher values are used).

Additionally, it can be seen that the zinc and acetic acid reduction has not affected the $^{3}H/^{14}C$ ratio.

In experiment B (p. 45) a much larger amount of all trans-squalene-11,14-$^{3}H_2$ was converted to sterols in the same fashion. Lanosterol was separated from the resulting mixture. It was con-

verted to its benzoate, which was oxidized with chromium trioxide. The oxidation product was methylated with diazomethane. The resulting methyl 25, 26, 27-trisnor-3-benzoyloxy-7, 11-diketo-Δ^8-24-lanostenoate (III) was purified by thin layer chromatography and two recrystallizations from ethanol.

To this material was added enough of the same compound, which was labelled with ^{14}C at positions 1, 7, 15, 22, and 31, to obtain a $^{3}H/^{14}C$ ratio of about 20. The material labelled with ^{14}C was obtained by enzymatically converting d, l-mevalonic-2-^{14}C acid to sterols. The lanosterol obtained from this preparation was treated in exactly the same way as that in the above preparation of specifically tritiated lanosterol.

After the $^{3}H/^{14}C$ ratio was established, cold material of the same high purity was added to give a workable amount of compound. This now doubly labelled material was reduced with zinc in acetic acid to compound IV. Compound IV was treated with phenylmagnesium bromide two times, since under the reaction conditions employed (refluxing in ether, instead of refluxing in benzene) the phenyl ketone [uv(methylene chloride)λ_{max} = 240mμ], which would be expected from the single addition of phenyl grignard, resulted. The resulting diphenyl carbinol was dehydrated with acetic anhydride/acetic acid to give a diphenylethylene compound, V, which was recrystallized twice from n-butanol.

The carefully purified diphenylethylene compound, V, was brominated with N-bromosuccinimide and dehydrobrominated with

N, N-dimethylaniline to give a diphenylbutadiene compound, VI. This material was also recrystallized twice from n-butanol.

The activities of tritium in these two compounds (V and VI were determined. The data from ten sequential 100 minute counts were combined to obtain the figures given (see p. for complete data).

Table 2	^{3}H(cpm/μm) ± 1σ	^{3}H(dpm/μm) ± 1σ
Diphenylethylene compound (V)	6.61 ± 0.16	20.5 ± 0.6
Diphenylbutadiene compound (VI)	6.61 ± 0.13	20.5 ± 0.5

The $^3H/^{14}C$ ratios are not reported due to the large counting errors for these quantities.

Amount of tritium at C-20:

$$\frac{6.61 \pm 0.16 - 6.61 \pm 0.13}{6.61 \pm 0.16} = 0.0 \pm 0.2 \text{ or } 0.0\% \pm 3.0\%$$

Fraction of tritium at C-14 which migrated to C-20:

$$\frac{6.61 \pm 0.16 - 6.61 \pm 0.13}{6.61 \times 0.348} \times 100 = 0.0 \pm 8.6\%$$

Maximum uncertainty due to the uncertainty in the amount of tritium at C-14:

$$\frac{0.20}{6.61} \times \frac{0.092}{(0.348)^2} \times 100 = 2.3\%$$

Total uncertainty fraction of tritium at C-14:

$$[(8.6\%)^2 + (2.3\%)^2]^{\frac{1}{2}} = 8.9\%$$

These data show that there could have been as much as $0.0 \pm 8.9\%$ of the tritium, located originally at C-14 in squalene, at C-20 in lanosterol. Thus, within the confidence levels given (i.e., 1σ) it can be said that 8.9% of the tritium from C-13 of the presterol cation could have migrated to C-20 of lanosterol. Therefore it appears that 91.1% of the tritium from C-13 of the presterol cation should have migrated from C-14 to C-17.

It is not unreasonable to assume that an enzymatically controlled reaction will be stereospecific. If this is the case, it seems most probable that the tritium originally at C-14 in the presterol cation has migrated to C-17 in lanosterol and therefore the C-18/C-19 double bond was trans just prior to cyclization.

An even more conclusive experiment could be conducted if enough doubly labelled (^{3}H and ^{14}C) material could be obtained so that the diphenylbutadiene compound (VI) could be oxidized to the keto compound (VII), from which the proton at C-17 could be exchanged, thereby quantitating the amount of tritium at this position.

IV. EXPERIMENTAL

A. Chemical degradation of lanosterol

1. Lanosteryl benzoate

Lanosterol (10 g, 23 mmol, Aldrich Chem. Co., recrystallized twice from acetone, mp 138-139.5°) was dissolved in 150 ml of benzene. The volume was reduced by one-third by distillation. To this was added 25 ml dry pyridine (distilled from barium oxide) and benzoyl chloride (25 ml, 0.276 mol). The solution was stirred overnight, after which most of the solvents were removed on a rotary evaporator. Water was added to destroy the excess benzoyl chloride. The product was extracted with three 100 ml portions of ether. The ether was washed repeatedly with water, 10% hydrochloric acid and saturated sodium bicarbonate solution. The ether layer was dried with three 30 ml portions of saturated sodium chloride solution and over magnesium sulfate. The ether was removed on a rotary evaporator. The product was recrystallized from methylene chloride-methanol. Yield: 4.5 g (36%), mp 182-186°, second crop, 2.2 g (18%), mp 180-185° [reported mp 191.5° (26)].

2. Methyl 25, 26, 27-trisnor-3-benzoyloxy-7, 11-diketo-Δ^8-24-lanostenoate (III) (27, 28, 29)

To a suspension of lanosteryl benzoate (4.5 g, 8.5 mmol) in 20 ml methylene chloride and 80 ml acetic acid was added over thirty minutes a solution of chromium trioxide (10 g, 100 mmol) in 100 ml of 90% acetic acid. The reaction was heated at 55° for three hours. Excess chromium trioxide was destroyed with 10 ml methanol. The

majority of the solvents was removed on a rotary evaporator. The products were taken up in 100 ml ether and 300 ml water. The water layer was extracted three times with 100 ml ether. The ether layer was washed repeatedly with 100 ml portions of water. The products were extracted with 10% sodium hydroxide solution. The basic extract was neutralized with concentrated hydrochloric acid and the product was extracted with three 100 ml portions of ether. The ether was dried with three 30 ml portions of saturated sodium chloride solution and dried over sodium sulfate. The ether was removed on a rotary evaporator. Yield: 1.5 g. A slurry of the acid in dry ether was treated with an ethereal solution of diazomethane, generated by treating N-nitroso-N-methylurea (1g, 10mmol) with 3 ml 40% potassium hydroxide solution in the cold and decanting the ethereal solution of diazomethane (30). After three hours, the excess diazomethane was destroyed with acetic acid. The ether was removed on a rotary evaporator and the product was recrystallized from ethanol. Yield: 0.404 g (8.5 %), mp 187-201°. Two further recrystallizations from ethanol gave 248 mg, mp 192-194.5°, uv (acetic acid) $\epsilon_{272} = 8,300$, ir (KBr pellet) C=O at 1675, 1710 and 1735 cm^{-1}, C—O—C at 1275 cm^{-1} and methyl ester at 1170 cm^{-1}. Analysis: 74.27% C, 8.31% H (Spang), calculated 74.43% C, 8.42% H.

3. Methyl 25, 26, 27-trisnor-3-benzoyloxy-7, 11-diketo-24-lanostanoate (IV) (31)

Methyl 25, 26, 27-trisnor-3-benzoyloxy-7, 11-diketo-24-

lanostenoate (III) (100 mg, 0.178 mmol) was dissolved in 5 ml acetic acid. The solution was heated to reflux. To this was added zinc dust (100 mg) and seven minutes later an additional portion of zinc dust (100 mg) was added. After fifteen minutes of reacting, the solution was filtered and the acetic acid removed on a rotary evaporator. The product was extracted in three 10 ml portions of ether. The ether was washed with three 5 ml portions of saturated sodium bicarbonate solution, dried with three 5 ml portions of saturated sodium chloride solution and dried over magnesium sulfate. The ether was removed on a rotary evaporator. Yield: 96 mg (95%). Recrystallization from ethanol gave material which had mp 212-215° [reported 220-221° (27)], ir (KBr pellet) C=O at 1695(s), 1708, and 1740 cm^{-1} and C—O—C at 1275 cm^{-1} and methyl ester at 1170 cm^{-1}.

4. Diphenyl carbinol derivative of IV

To a solution of methyl 25, 26, 27-trisnor-3-benzoyloxy-7, 11-diketo-24-lanostanoate (IV) (295 mg, 0.52 mmol) in 15 ml dry benzene was added 1 ml of an ether solution of phenyl magnesium bromide (3 mmol, 3 M, Arapahoe). This reaction was heated under reflux for five hours. The salts were decomposed with ice and 20 ml water. The product was extracted with three 20 ml portions of ether. The organic phase was dried with three 10 ml portions of saturated sodium chloride solution and then over magnesium sulfate. The solvents were removed on a rotary evaporator. The products were chromatographed on a 40 g silica gel column. The fractions eluted with 5% ether in

benzene contained the desired product. The eluant from these fractions was removed on a rotary evaporator. After recrystallization from ethanol, there was obtained 140 mg (39%), mp 200-210°, ir (KBr pellet) O—H at 3460 cm^{-1}, C=O at 1695, 1700(s), and 1715(s) cm^{-1}, C—O—C at 1275 cm^{-1} and aromatic bands at 680, 695, 705, 710, and 755 cm^{-1}.

An alternate preparation for the diphenyl carbinol derivative of IV, used in Experiment B (see p.), is described here.

To a solution of methyl 25, 26, 27-trisnor-3-benzoyloxy-7, 11-diketo-24-lanostanoate (IV) (179 mg, 0.32 mmol) in 8 ml dry benzene, from which about 12 ml benzene had been distilled, was added 20 ml dry ether and 0.3 ml phenylmagnesium bromide (3 mmol, 3M, Arapahoe). This reaction was heated under gentle reflux for one hour. The salts were decomposed with 5% sulfuric acid. The product was extracted in three 10 ml portions of ether. The organic phase was washed with saturated sodium bicarbonate and saturated sodium chloride. The solvents were removed in a stream of nitrogen.

The product was applied to two thick layer chromatography plates (silica gel F_{254}, Brinkmann, 20 cm x 20 cm x 2 mm), which were developed in 10% ether in benzene. The product (rf ~ 0.13) was scraped from the plates and eluted with acetone. Yield 144 mg (76%), uv(methylene chloride)λ_{max} = 242. This was the phenylketone which resulted from the single addition of phenylmagnesium bromide to IV.

Of this material, 100 mg (0.17 mmol) was dissolved in 15 ml dry ether. To this was added 0.3 ml phenylmagnesium bromide. The

reaction was heated under gentle reflux for one hour. The salts were decomposed with 5% sulfuric acid. The product was extracted with three 15 ml portions of ether. The ether was dried with three 10 ml portions of saturated sodium chloride. The ether was evaporated in a stream of nitrogen. The resulting product was used successfully to prepare the diphenyl ethylene derivative of IV.

5. Diphenyl ethylene derivative of IV

A solution of the diphenyl carbinol derivative of IV (140 mg, 0.204 mmol) in 11 ml of acetic acid-acetic anhydride (10:1) was heated under reflux for one hour. Five ml water was added and the solution was allowed to stand for 2 hours. The product was extracted with three 10 ml portions of ether. The ether layer was washed with three 10 ml portions of saturated sodium bicarbonate solution, dried with three 10 ml portions of saturated sodium chloride solution and dried over magnesium sulfate. The ether was removed on a rotary evaporator. The product was recrystallized twice from n-butanol. Yield: 60 mg (44%), mp 243-248° [reported 286-287° (27)], ir (KBr pellet) C=O at 1695 and 1715 cm^{-1}, C—O—C at 1272 cm^{-1} and aromatic bands at 695, 700, 710, 760, 770, and 800 cm^{-1}, uv (methylene chloride) maximum at 252 mμ [reported uv (chloroform) maximum at 255 mμ (27)]. This material was homogeneous by thin layer chromatography.

6. Diphenyl butadiene derivative of IV

To a solution of the diphenyl ethylene derivative of IV (30 mg, 0.045 mmol) in 5 ml carbon tetrachloride was added N-bromosuccini-

mide (9 mg, 0.050 mmol, MCB). The solution was heated and irradiated with a 150 watt light bulb for thirty minutes. The reaction was cooled and the succinimide was filtered from the solution. Carbon tetrachloride was removed on a rotary evaporator.

Two ml N, N-dimethylaniline was added to the flask and the reaction was heated at 70° for ten minutes. Water (10 ml) was added to the reaction and the products were extracted with three 10 ml portions of ether. The ether layer was washed with three 10 ml portions of 10% hydrochloric acid, dried with three 5 ml portions of saturated sodium chloride solution and dried over magnesium sulfate. The ether was evaporated on a rotary evaporator. The product was recrystallized from n-butanol. Yield 10 mg (33%), mp 253-258° [reported mp 236-237° (27)], uv (chloroform) maximum 310 mμ [reported uv (chloroform) maximum 310 mμ (27)]. This product was homogeneous by thin layer chromatography.

7. 22, 23, 24, 25, 26, 27-Hexanor-3-benzoyloxy-lanosta-7, 11, 20-trione (VII)

To a solution of the diphenyl butadiene derivative of IV (10 mg, 0.015 mmol) in one-half ml methylene chloride and 3 ml acetic acid was added a solution of chromium trioxide (20 mg, 0.20 mmol) in one ml 90% acetic acid. The reaction was heated at 55° for 2 hours. The excess chromium trioxide was destroyed with methanol and the solvents were removed on a rotary evaporator. Water (10 ml) was added to the reaction. The product was extracted with three

15 ml portions of ether. The ether was washed with two 10 ml portions of water, two 10 ml portions of saturated sodium bicarbonate solution, dried with three 10 ml portions of saturated sodium chloride solution and dried over magnesium sulfate. The solvent was removed on a rotary evaporator. The residue was applied to a thin layer chromatography plate, which was then developed with 5% ether in benzene. A band with an rf of 0.1 contained 2.2 mg (0.0045 mmol, 30% yield) of the desired product. This material had a uv (methylene chloride) maximum at 230 mμ and a very small uv maximum between 280 and 290 mμ [reported uv (chloroform) maximum at 295 mμ (27)]. The ir (KBr pellet) of this material showed C=O at 1705 cm^{-1} (very broad), C—O—C at 1272 cm^{-1} and an aromatic band at 710 cm^{-1}.

B. Chemical synthesis of all trans-squalene-11,14-$^3H_2$

1. Tetramethylene-1,4-bistriphenylphosphonium dibromide (32)

To a 250 ml. flask, equipped with reflux condenser and drying tube, was added 90 ml 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° (5 mm)]. The reaction was heated under reflux for two days, after which the solids were dissolved in hot chloroform and precipitated with ether. The product was dried under vacuum at 180° over phosphorous pentoxide for two days. The recovery was 57.7 g, mp 298-300° (reported mp 296-298° (32)].

2. Tertiary-butyl alcohol-1-^{3}H

To dry t-butyl alcohol (about 15 ml, distilled from calcium hydride) was added tritiated water (about 500 mc in about 0.1 ml, Nuclear Chicago). After two hours, calcium hydride (2 g, 47.7 mmol) was added to the solution. The solution was heated under reflux for two and one-half hours, after which the t-butyl alcohol-1-^{3}H was distilled into a 100 ml three necked flask. Yield: about 12 ml.

3. Squalene-11, 14-$^3H_2$ (24, 25)

To the dry tritiated t-butyl alcohol was added potassium (320 mg, 8.2 mmol). After six hours the potassium had reacted completely and tetramethylene-1, 4-bistriphenylphosphonium dibromide (2.8 g, 3.8 mmol) was added to the solution. The flask was flushed with nitrogen and then sealed . After sixteen hours, trans-geranyl acetone (1.61 g, 8.4 mmol, 99+ % trans by vpc analysis) was added to the reaction. After five hours, a small portion of the reaction medium was removed and diluted to determine the specific activity of t-butyl alcohol, sp. activity = 5.55 mc/mmol. The reaction was quenched by addition of water (15 ml). The aqueous phase was extracted with three 15 ml portions of ligroin (30-60°). The ligroin phase was washed with three 10 ml portions of water, dried with three 10 ml portions of saturated sodium chloride solution and dried over sodium sulfate. Yield: 48.6 mc.

The product was applied to a chromatography column of Woelm activity II neutral alumina (60 g). The first four fractions,

ligroin (30-60°), contained squalene. These fractions were combined and concentrated. Yield: about 1.5 g. This material was divided into four parts, each of which was applied to a thin layer chromatography plate (10 cm by 20 cm by 2 mm, Brinkmann, silica gel F_{254}). The plates were developed in benzene-ligroin (1:1). The squalene bands (rf 0.7) were scraped from the plates and eluted with ether, acetone, and methanol. The solvents were evaporated in a stream of nitrogen. Yield: about 600 mg.

To the flask containing the squalene was added 100 ml methanol, saturated with thiourea, and thiourea (1 g, powdered). After the solution had been stirred in a cold room (4°) for four days, the solid thiourea clathrate was filtered from the solution and it was washed with three 5 ml portions of cold methanol (33).

The thiourea clathrate was decomposed with water (75 ml) and the squalene was extracted with three 30 ml portions of ligroin (30-60°). The ligroin layer was dried with three 20 ml portions of saturated sodium chloride solution and over sodium sulfate. Yield: 4 mc.

C. Enzyme and biological preparations

1. Preparation of rat liver homogenate (16)

Livers were excised from male Sprague-Dawley rats (130-165 g), shortly after they had been sacrificed by a blow on the

head.* The livers were stored on ice until they were taken to a cold room (4°C), where they were homogenized, usually within thirty minutes of the time when the rat was sacrificed. The livers were forced through a stainless steel frit, which contained about 50 holes of 1.5 mm diameter (34). This material was further homogenized in a teflon and glass tissue homogenizer (0.006-0.009 inch 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 cheesecloth. Yield: about 15 ml enzyme preparation, S_{12}, for 10 g of liver.

2. Preparation of labelled squalene (35)

Forty ml of S_{12} liver homogenate was placed in a 250 ml Erlenmeyer flask. Nitrogen was passed through the homogenate until the oxygen content was less than 5% of that in air saturated water, as determined with a YSI Oxygen Monitor. For the duration of the reaction time, a slow stream of nitrogen was passed through the solution. To the enzyme preparation was added d, l-mevalonic-2-^{14}C acid (2 to

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*It was observed that homogenate prepared from older rats (400-500 g) were more active for synthesis of squalene, but not for synthesis of sterols.

3 mg, 11.5 to 17 μc, Tracerlab) as its N, N'-dibenzylethylene diamine salt. Each of the following cofactors was added at time = 0, 1, and 2 hours, adenosine triphosphate (59 mg, 0.1 mmol), nicotine adenine dinucleotide, reduced (7 mg, 0.01 mmol), nicotine adenine dinucleotide phosphate (4 mg, 0.005 mmol), and glucose-6-phosphate (2 mg, 0.0067 mmol), and at time = 0, sodium fluoride (8.4 mg, 0.5 mmol). After three hours incubation on a shaker in a 37° room, the reaction was stopped by the addition of 80 ml 15% potassium hydroxide in 50% ethanol-water. The mixture was hydrolyzed for three hours at 70°. After the hydrolysate was cooled to room temperature, it was extracted with three 50 ml portions of ligroin (30-60°). The ligroin layer was washed with two 25 ml portions of water, dried with three 25 ml portions of saturated sodium chloride solution and dried over sodium sulfate. The ligroin was removed on a rotary evaporator. Yield: 3.4 μc (about 50% for the d form). The total product was chromatographed on a 2 g column of Woelm activity II neutral alumina. The first fraction (ligroin, 10 ml) contained squalene (1.3 μc) and the second fraction (acetone-ether 1:1, 10 ml) contained sterols (2.1 μc).

3. Preparation of sterols from labelled squalene (20)

A sample of biosynthetic squalene-^{14}C (derived from d, l-mevalonic-2-^{14}C acid) (25,000 dpm, about 1.2 mμm or about 0.5 μg) was added to a 25 ml test tube. The solvent was evaporated in a stream of nitrogen. To this was added lanosterol (100 μg), 1,2-propanediol (20 μl) and acetone (50 μl). Rat liver homogenate (5 ml) was added to

the sample. Each of the following cofactors was added at time = 0, 1, and 2 hours, nicotine adenine dinucleotide phosphate (2.2 mg, 0.0029 mmol) and glucose-6-phosphate (4.5 mg, 0.015 mmol). The sample was placed on a shaker in a 37° room for three hours, after which time it was quenched by the addition of 10 ml 15% potassium hydroxide in 50% ethanol-water. The hydrolysis was carried out at 70° for two hours. After the hydrolysate had been cooled to room temperature, it was extracted with three 5 ml portions of ligroin (30-60°). The ligroin layer was dried with three 5 ml portions of saturated sodium chloride solution and over sodium sulfate. The products were separated by thin layer chromatography on analytical plates (5 cm by 20 cm by 0.25 mm, Brinkmann, silica gel F_{254}), developed in 20% ether in benzene. Yield: lanosterol (6,000 dpm, 24%), cholesterol (10,000 dpm, 40%).

D. Materials and methods

1. Scintillation counting

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

Two different scintillation solutions were employed. For analytical work, when samples were checked for purity by thin layer chromatography, samples, which were still deposited on silica gel,

were placed in Bray's solution, a dioxane based solution (36). For all other work, the 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 toluene to one liter [final solution contained 2, 5-diphenyloxazole (PPO, 5g/l) and 1, 4-bis-(4-methyl-5-phenyloxazoyl)-benzene (dimethyl POPOP, 0.1 g/l)].

To determine $^{3}H/^{14}C$ ratios, the data obtained from the scintillation counter were analyzed on an IBM 7090/7094 computer. The data were analyzed to determine the absolute number of disintigrations per minute (dpm) for each isotope and the ratio of these numbers. Furthermore, the statistical error in each number was determined and the error in the $^{3}H/^{14}C$ ratio was determined by calculation of the root mean square of the errors for additive operations and of the percent error for multiplicative operations.

2. Chemicals

Adenosine-5'-triphosphate, from equine muscle--Sigma Chemical Co.

Glucose-6-phosphate disodium salt--Calbiochem.

Nicotine-Adenine dinucleotide phosphate monosodium--Calbiochem.

Nicotine-Adenine dinucleotide reduced disodium--Calbiochem.

d, l-Mevalonic-2-^{14}C acid, dibenzylethylene diamine salt--Tracerlab.

d, l-Mevalonic-5-^{3}H acid--Tracerlab.

Lanosterol--Aldrich Chemical Co.

E. Data

Data for Experiment A

	Wt. (mg)	^{3}H(dpm/mg)	$^{3}H/^{14}C$
Squalene-11, 14-$^{3}H_2$ + squalene-1, 5, 9, 16, 20, 26-$^{14}C_6$			22.6 ± 0.7
Lanosterol-^{3}H, ^{14}C crystallized once		126 ± 5	16.8 ± 1.3
Supernatant from above crystallization		370 ± 15	16.8 ± 1.4
Lanosterol-^{3}H, ^{14}C crystallized twice		97 ± 4	19.6 ± 2.3
Supernatant from above crystallization		89 ± 5	16.8 ± 4.6
Lanosteryl benzoate (60 mg lanosteryl benzoate added)	39.7		22.0 ± 2.3
Compound III	33.4		24.0 ± 2.2
Compound III + III - ^{14}C (67 mg unlabelled compound III added)			12.0 ± 2.2
Compound IV	100		14.2 ± 0.8

Data for Experiment B

	^3H(dpm)	Wt.(mg)	$\frac{^3H(dpm)}{mg}$	$\frac{^3H(dpm)}{\mu m}$
Squalene-11,14-$^3H_2$	1.8×10^9			
Sterols	2.6×10^7			
Lanosterol	2.4×10^6			
Lanosteryl benzoate	1.3×10^6	107	1200	
Lanosteryl benzoate + unlabelled lanosteryl benzoate	1.3×10^6	507	384	
Compound III	5.5×10^5	150	366	
Compound III, crystallized	1.1×10^4	80	139±3	
Compound III, recrystallized	8.1×10^3	71	115±4	
Compound III + unlabelled Compound III	8.1×10^3	201	40.3±1.3	22.7±0.7
Compound IV	7.0×10^3	187	38.7±0.6	21.9±0.3
Compound V	500	16.7	30.6±0.9	20.5±0.6
Compound VI	100	3.5	30.6±0.7	20.5±0.5

Counting data (100 minute counts) used to determine the specific activities of compounds V and VI.

Count Number	Blank	Compound V	Compound VI
1	1125	1814	2093
2	1122	1883	(1953)
3	1115	1886	2027
4	1142	1898	2049
5	1145	1871	2023
6	1096	1889	2094
7	1127	1894	2007
8	1089	1906	2068
9	1134	1817	2028
10	1128	1851	2022
11	-	-	2108
	11223	18709	20519
Average	1122	1871	2052
Sum of deviation^2	2957	9741	11813
Average deviation	296	974	1181
Standard deviation	18.1	32.9	34.4

Note: Count # 2 for Compound VI was not used since it was 2.88 standards from the mean.

Specific activities of compounds V and VI.

	Gross Counts	Net Counts	Sample Weight	Counts / mg
Compound V	18709 ± 137	7486 ± 173	0.76 mg	9850 ± 233
Compound VI	20519 ± 143	9296 ± 178	0.94 mg	9889 ± 196

	Counts / μm	CPM / μm	DPM / μm
Compound V	6606 ± 156	6.61 ± 0.16	20.5 ± 0.6
Compound VI	6612 ± 131	6.61 ± 0.13	20.5 ± 0.5

Note: The efficiency of tritium counting was 32.28% ± 0.46%. Although, theoretically, there were a few ^{14}C counts, there were so few (less than 0.2 cpm in the tritium channel, where the ^{14}C efficiency was 17%) that they were neglected for these calculations. Since the counting efficiency in the two samples, as measured by the automatic external standardization unit of the Packard Scintillation Counter was the same, it is sufficient to compare the CPM/μm, where the error is somewhat less, since the error in determining the tritium counting efficiency does not appear in these figures. An error of 0.5% in the sample weight was allowed, which is greater than the 2.5 μg error (5 mg range) specified by the manufacturer of the balance, Cahn.

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VI. PROPOSITIONS

Abstracts of Propositions

I. It is proposed that 1, 2-(α-ketotetramethylene)ferrocene, which can be resolved into d and l forms, be investigated as a photochemical sensitizer which might induce residual optical activity in a racemate by causing the d and l forms to undergo photochemical transformations at different rates.

II. An epoxide is proposed as an intermediate in the enzymatic oxidation of β-carotene to retinal.

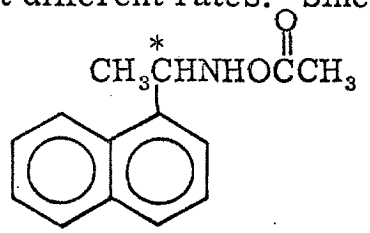
III. It is proposed that the mechanism of the opening of the 9, 19-cyclopropane ring of cycloartenol be investigated by observing the fate of a tritium label, which would be placed at C-8 by an enzymatic synthesis.

IV. It is proposed that the mechanism of squalene cyclization be investigated by preparation of 20, 21-dehydrosqualene and its enzymatic conversion to tetracyclic compounds.

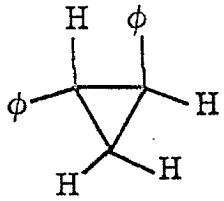
V. Steroids, which are prepared from specifically deuterated squalenes, are proposed as substances for the further investigation of the mass spectra of steroids.

PROPOSITION I

In 1965 Hammond and Cole (1) showed that an optically active sensitizer (I) could induce residual optical activity in a racemate (II), by causing the d and l isomers to undergo photochemical transformations at different rates. Since that time, there has been no report of



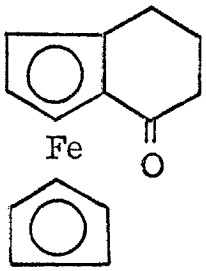
I



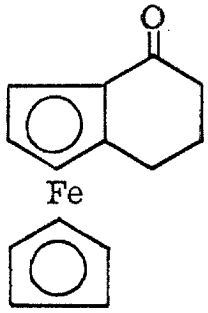
d,l-trans-1, 2-diphenylcyclopropane (II)

any other sensitizers which have been used to the same end successfully.

It is proposed that a large number of compounds derivable from 1, 2-(α-ketotetramethylene)-ferrocene (III), which is resolvable into d and l forms (2), be investigated as photochemical sensitizers. This



(+) - III



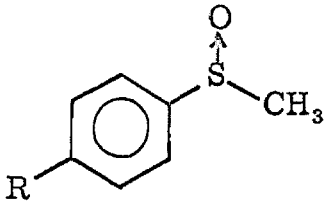
(-) - III

compound offers an unusual variety of optically active derivatives, obtainable without risk of stereochemical inversion.

Ferrocene has been shown to sensitize the cis-trans isomerization of piperylenes and there is some evidence that this isomerization

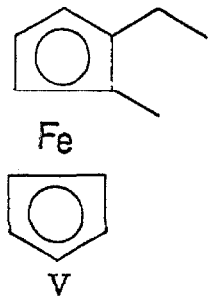
proceeds via a complex between the sensitizer and substrate (3, 4, 5). Furthermore, there is crystallographic evidence that, in the complex between tetracyanoethylene (TCNE) and ferrocene, TCNE is planar to and situated above one of the five-membered rings of ferrocene (6). Therefore, it is possible that other complexes would form in this fashion (rather than directly to the d-orbitals of the iron atom), thereby facilitating close interaction between the assymetric center of III and a substrate (e.g., II).

Although complexes may form to both rings of ferrocene, to the extent that one forms to the substituted ring, some induction of optical activity via photochemical sensitization may be observed. Additionally, such a substrate-sensitizer complex has been postulated in the sensitized stereochemical racemization of IV by naphthalene (7).



IV R = CH_3, Br, OH

Even if the parent compound (III) proved to be an inefficient sensitizer, it would be possible to prepare other derivatives [e.g., V(2)], which might be more suitable for such investigations.



V

Although it might seem that for many photochemical reactions there is insufficient energy available from the first triplet of ferrocene (about 43 kcal/mol) (3), there has been a report of an emission from higher excited states of ferrocene (5, 10). More importantly, ferrocene sensitizes reactions (3) which require about 60 kcal/mol (3). Therefore, it seems possible that the proposed compounds (III, V and related compounds) might serve as sensitizers, which could be used to further investigate the interactions between sensitizers and substrates in photochemical reactions.

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PROPOSITION II

DeWitt S. Goodman has published a series of articles about β-carotene (I), retinal, retinoic acid, vitamin A (II), the biosyntheses of these compounds and their biological distributions (1-7). A proposed mechanism for the biological conversion of β-carotene to retinal has been supported in this work (4).

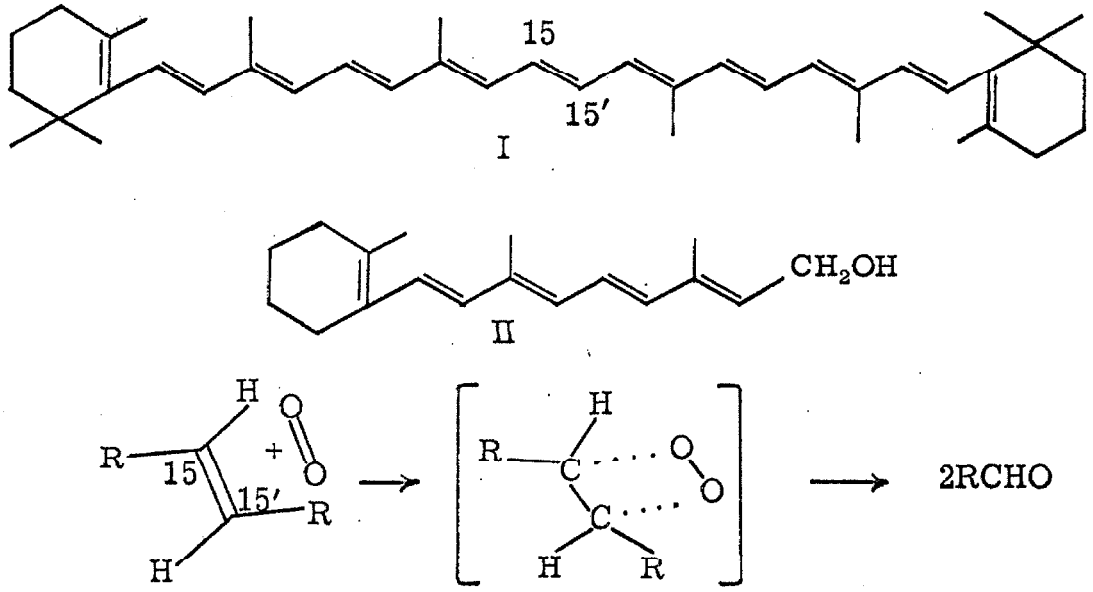
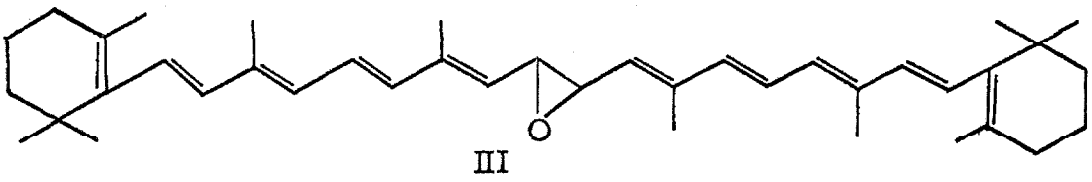


Figure 1. Goodman's Mechanism (4)

Purification of the enzyme responsible for this oxidation was not possible (6).

It is proposed that an intermediate epoxide (III) exists and that more than one enzyme is required for the conversion of β-carotene to retinal. Further, it is proposed that 15, 15′-oxide-β-carotene be synthesized by the method outlined (8) in Figure 2, and that this



III

compound be tested for its biological activity.

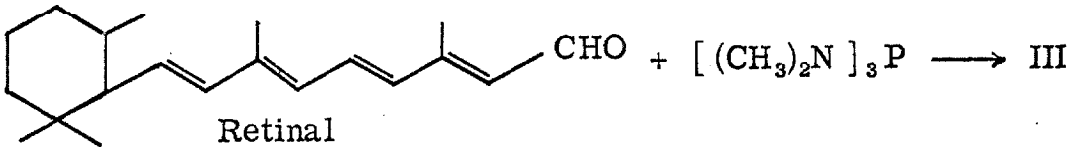


Figure 2

References

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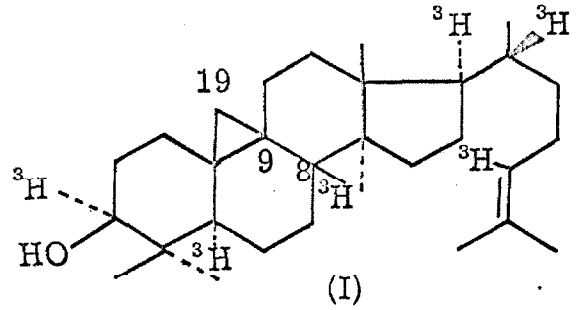
PROPOSITION III

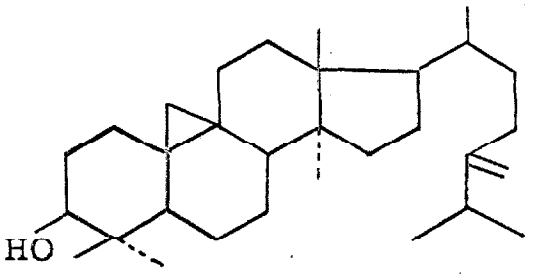
Cycloartenol (I) serves as the common intermediate between trans-squalene-2, 3-oxide and plant sterols (1-4). However, there is a major structural distinction between cycloartenol and most phytosterols, that being the presence of a 9, 19-cyclopropane ring.



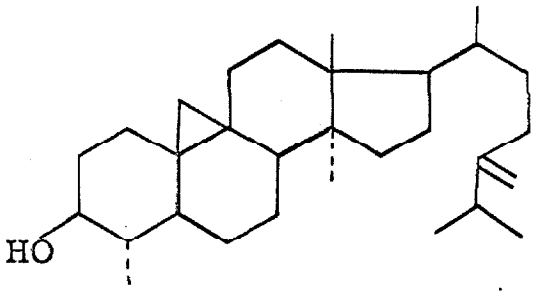
(I)

It is known that cycloartenol, biosynthesized from mevalonic-4-R-4-^{3}H, 2-^{14}C acid, retains six tritium labels (5). One of these labels is at C-8 (5).

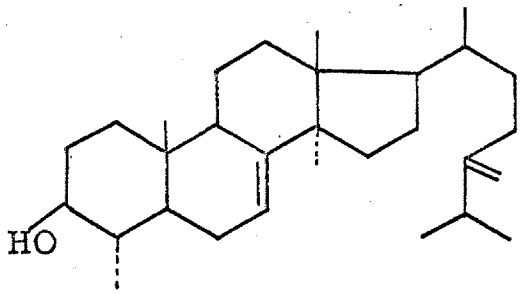
It is proposed that the mechanism of the opening of the 9, 19-cyclopropane ring be investigated by observing the fate of the tritium at C-8. This can be readily done by studying ^{3}H/^{14}C ratios in other phytosterols (II-V), which can be obtained from the same sources as cycloartenol (6).



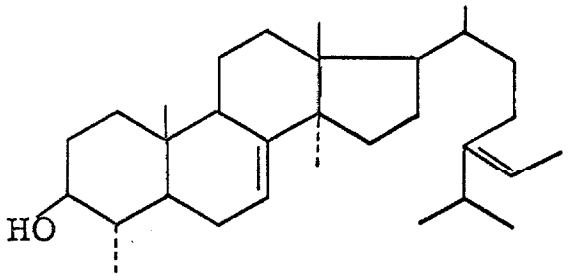
24-Methylenecycloartenol (II)



24-Methylenecycloeucalenol (III)



24-Methylenelophenol (IV)



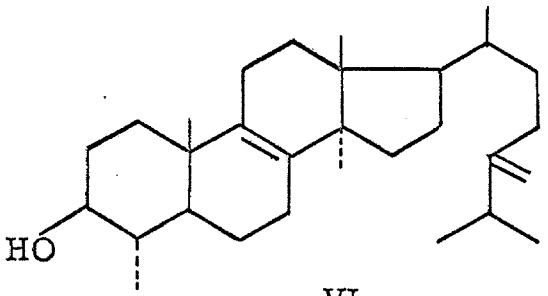
24-Ethylidenelophenol (V)

In the lophenols, it might be expected that the tritium from C-8 would be at C-9 (via 1, 2-migration), at C-19 (via intra- or intermolecular transfer) or to be absent from the product (via elimination or abstraction).

If the label remains in the product, oxidation with N-bromosuccinimide to the 7, 9(11)-diene would show whether the label were at C-9. This is not an unexpected possibility, since acid treatment of cycloartenol opens the cyclopropyl ring, generating a cationic center at C-9.

Presence of the label at C-19 requires a highly strained conformation for proton transfer, and therefore is unexpected.

Absence of this label might indicate that a new intermediate (VI) exists.



VI

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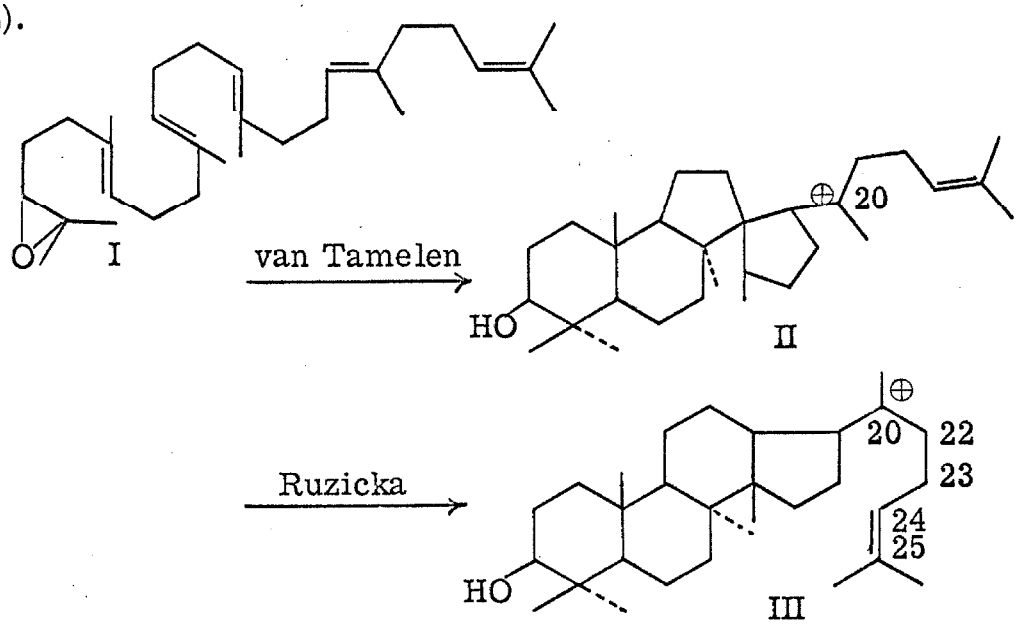
PROPOSITION IV

The most recently proposed mechanism for the cyclization of squalene-2, 3-oxide (I) is the one of van Tamelen (1), in which a spiro intermediate cation (II) is supported instead of Ruzicka's cation (III) (2).



It is proposed that 20, 21-dehydro-squalene (IV) be synthesized and converted enzymatically in a stepwise fashion to its terminal epoxide and its cyclized product (V or VI).

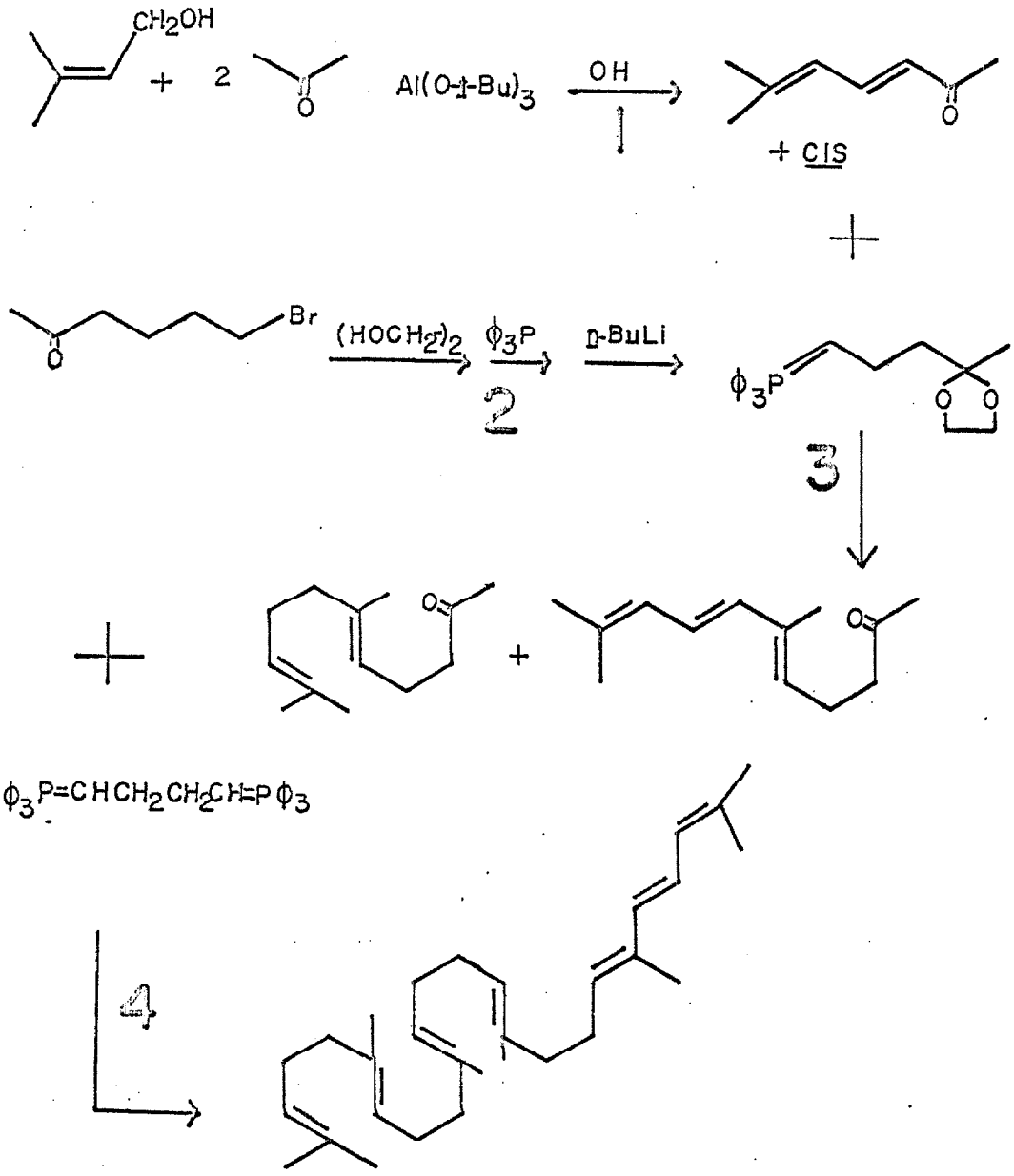
As C-20 becomes a full cation, it is quite probable that the charge would become delocalized over the available π-system (C-22 to C-25) of the substituted butadiene. Quenching of this cation at C-25 by water would lead to a neutral product (V or VI).

If VI were the observed product, then van Tamelen's proposal would be strongly indicated. However, if V were the observed

product, it would seem that Ruzicka's original proposal was correct.

In the proposed synthesis, reaction 1 has been reported previously (3). 5-Bromo-2-pentanone is readily available (4). Compounds which contain ketone functions and bromine can be converted to ketals without affecting the bromine (5), and ketals are not affected by Wittig reactions (6).

Although step three of the proposed synthesis of IV will generate cis and trans isomers, it is possible that the cis isomer will cyclize to a cyclohexadiene, making it readily separable from the trans isomer. However, the inclusion of some cis isomer would not be a serious drawback, since it is expected that either bond isomer in the final product would be usable (7).

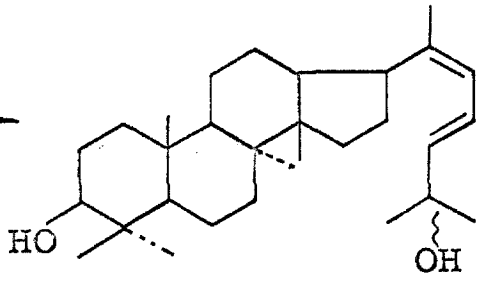

CH2OH
+ 2
Al(O-t-Bu)3
OH
+ CIS
Br
(HOCH2)2
φ3P
n-BuLi
2
3
φ3P=CHCH2CH2CH=Pφ3
4

IV

Figure 1

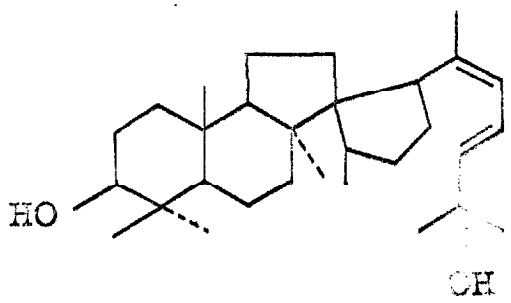
IV + squalene-2, 3-oxide $\xrightarrow[\text{rat liver homogenate}]{\text{aerobic incubation with}}$ IV-2, 3-oxide

IV-2, 3-oxide $\xrightarrow[\text{rat liver homogenate}]{\text{anaerobic incubation with}}$



V

or



VI

Figure 1 (continued)

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PROPOSITION V

The mass spectra of steroids has been a topic of interest in the past few years. Thus far the mass spectra of steroids have been used as fingerprints for these compounds. The major problem in analyzing the mass spectrum of a steroid is the lack of specific knowledge concerning fragment formation during ionization. The bulk of information about fragmentation has been obtained by qualitative comparison of mass spectra of known compounds of similar structure (1, 2, 3, 4).

Keto-steroids have been reasonably well investigated by substituting deuterium at exchangeable positions in the molecule by Djerassi and co-workers and others (5, 6, 7, 8, 9, 10, 11, 12, 13). Analysis of the spectra of these deuterated keto-steroids has allowed Djerassi to make considerable inroads into the problem of fragment formation. However, the important field of steroidal alcohols has been virtually untouched, except for comparison of spectra of similar compounds. Many interesting problems can be solved by using properly labelled steroids, but due to lack of suitable functionality, deuteration is not easy and requires much more chemistry than is necessary with steroidal ketones.

Since the discovery of the intermediacy of 2, 3-oxido-squalene in the conversion of squalene to lanosterol (14), it is much easier to control the products obtained; for example, by using an anaerobic incubation, only lanosterol will be obtained, even with a crude enzyme preparation.

It is proposed that numerous deuterated squalenes and their 2, 3-oxides be prepared and be converted enzymatically to lanosterol. The mass spectra of such specifically deuterated steroids would permit many problems associated with fragment formation to be solved. Many positions in the steroid nucleus are available for deuteration, via synthetic squalene (Figure 1).

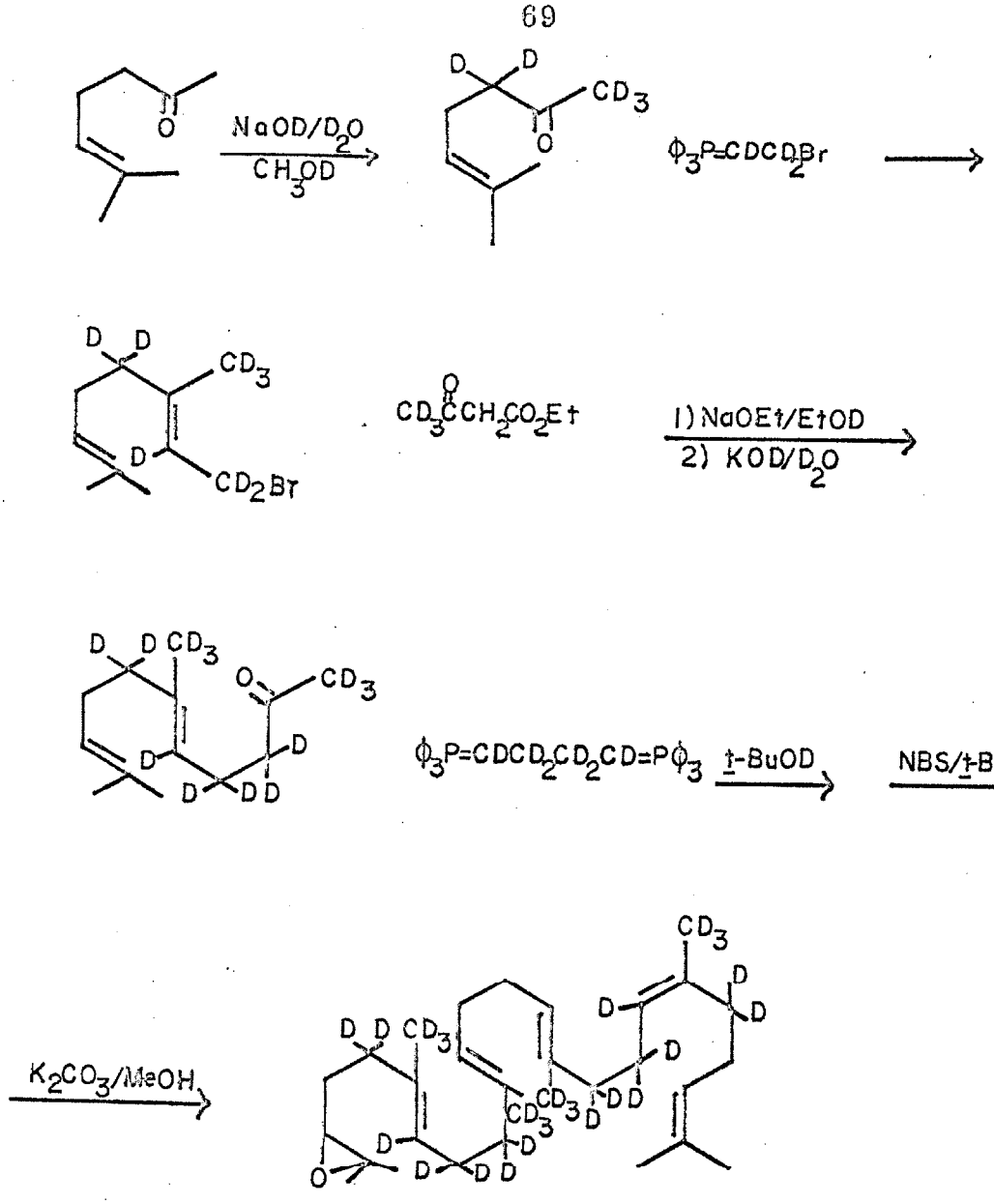
NaOD/D2O
CH3OD
Φ3P=CDCD2Br
CD3CCH2CO2Et
1) NaOEt/EtOD
2) KOD/D2O
Φ3P=CDCD2CD2CD=PΦ3
t-BuOD
NBS/t-BuOH
K2CO3/MeOH

Figure 1

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