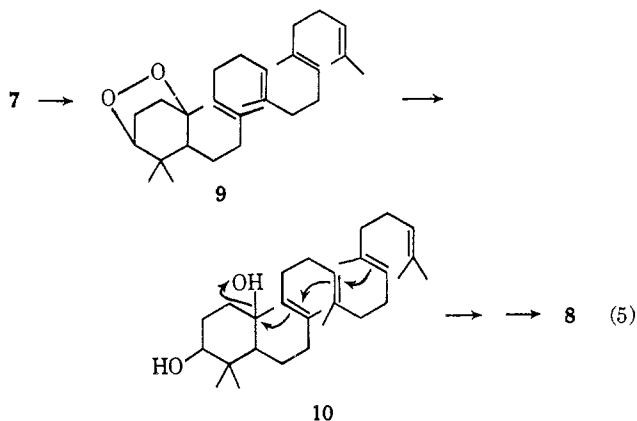


has been made for enzymatic hydroxylation at C-3 of a preformed lanostadiene system (eq 4).⁶ Still more recently, consideration has been given to an initiation process involving oxidative cyclization by molecular oxygen to a bridged peroxide (**9**) followed by reductive cleavage to a monocyclic diol (**10**) (eq 5). Cyclization of derived carbonium ion would complete the conversion to lanosterol.⁷

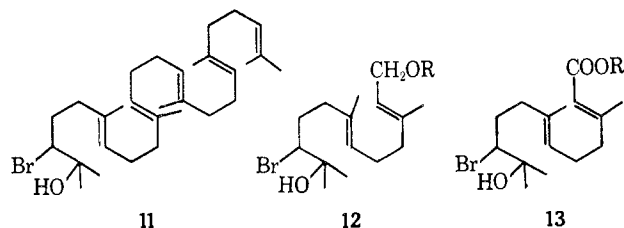


In the hope of shedding light on this area, we initiated during the early 1960's organic chemical investigations, the results of which suggested that sequential epoxidation and cyclization processes might be involved in the biochemical conversion of squalene to polycyclic terpenoids. This stepwise mechanism differs from all other biosynthetic proposals and later was confirmed

by enzymic experiments which demonstrated both the efficient anaerobic conversion of squalene 2,3-oxide to lanosterol and the transformation of squalene to its 2,3-oxide in the presence of oxygen. Accordingly, a detailed chemical examination of terpene epoxidation and cyclization is of considerable importance from the biochemical point of view.

Given the problem of investigating from the organic chemical point of view the oxidation-cyclization of squalene to lanosterol, one must consider at least four separate but related aspects: (1) the oxidation of squalene (or a model system) at the terminal olefinic position; (2) conversion to polycarboyclic intermediates, with provision for appearance of hydroxyl at C-3; (3) stereochemical aspects of the cyclization process; and (4) the generation of lanosterol *per se* from polycyclic precursors, concluding with the established manifold hydrogen-methyl migration sequence and generation of the $\Delta^{8(9)}$ double bond.

Since squalene and most acyclic terpenes possess only nonconjugated, trisubstituted double bonds which are approximately chemically and sterically equivalent, the selective oxidation of the terminal olefinic link represents an unusual problem which, as far as we can determine, had found no solution in past work. On the basis of considerations to be taken up later, we attempted at an early stage selective oxidation of squalene in a highly polar, aqueous-organic medium with the oxidizing agent N-bromosuccinimide (NBS).⁸ In this case, as well as in the farnesyl acetate or ether, farnesic ester, and other cases, highly selective attack (>95%) occurred, providing terminal bromohydrin (**11-13**). Similarly, either mercuric acetate or aqueous HCl effected hydration at the terminal site to the virtual exclusion of reaction at the internal olefinic centers.

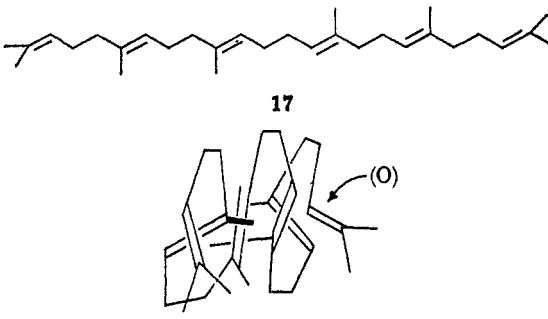


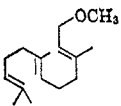
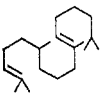
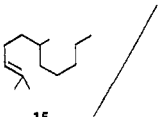
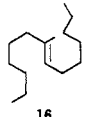
As a means of investigating the selectivity phenomena, three principal variations were introduced in the oxidation experiments: the structure of the olefinic substrate, the nature of the medium, and the type of oxidizing agent.^{8,9} In Table I are summarized some of the results which suggest that the *in vitro* selectivity is due to both steric and conformational characteristics of the olefinic system. A high order of selectivity is observed not only with sesqui- and higher terpenes, but also with purely synthetic cases (*e.g.*, **14**) where the double bonds are more widely separated. Similarly,

(6) D. H. R. Barton and G. P. Moss, *Chem. Commun.*, 261 (1966).
 (7) A. H. Soloway, *J. Theoret. Biol.*, **13**, 429 (1966).

(8) E. E. van Tamelen and T. J. Curphey, *Tetrahedron Letters*, 121 (1962).
 (9) E. E. van Tamelen and K. B. Sharpless, *ibid.*, 2655 (1967).

Table I
Conformational-Steric Effects in Formation of
Bromohydrins by Action of NBS



Olefins	Glyme-water		Petr ether-acetic acid	
	Term, %	Int, %	Term, %	Int, %
	96	4 (allylic = 0)	81	19 (allylic = 0)
	98.5	1.5	62	38
	100	0
				

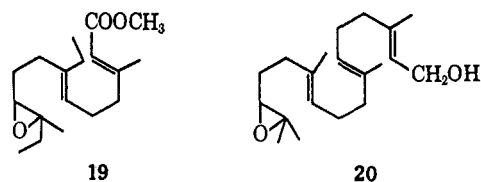
in a competitive reaction involving two monoolefins, one with a "terminal" double bond (15) and the other with an "internal" link (16), the former is oxidized to the virtual exclusion of the latter. As expected, various kinds of terminal trisubstituted double bonds exhibit little difference in susceptibility to attack. Selectivity as exemplified in Table I *must* be due *in part* to the more adverse steric effects operative in the environment of the internal double bond(s). However, that other factors contribute is indicated by the facts that: (1) most oxidizing agents do not exhibit terminal selectivity, which would be expected if the effects were only steric;⁸ and (2) a pronounced solvent effect has been observed (Table I).⁹ In petroleum ether-acetic acid, selectivity falls to as low a value as 62%. Similar, although less dramatic, differences are observed in certain peracid oxidations, a much less preferential process in general.⁹

Although the effect of solvent on selectivity is subject to various interpretations, it most likely is related to the conformation of the olefin in solution. In a solvent of low polarity there is no reason to suppose that, for example, squalene would not exist for the most part in an uncoiled, fully extended state (17), with all of the double bonds equally vulnerable to attack by an oxidizing agent. On the other hand, in a more polar medium (such as that offered by a hydroxylic solvent) it appears possible that squalene would assume a more highly coiled, compact conformation (18), such that the triterpenoid would be "internally solvated," and the system of hydrogen bonds

in the medium would be disrupted as little as possible. Should this be the case, the internal double bonds in this coiled conformation might be sterically shielded and thus chemically less reactive, whereas the terminal olefinic links would remain exposed. If the stereo-electronic requirements for bromonium ion attack are now superimposed on the above, the geometrical constraints on the oxidation process become even more rigid, and the vanishingly small involvement of the internal olefinic bonds becomes quite understandable. The Curtin-Hammett principle¹⁰ is not violated, since the energy increment ascribed to solvent influence could be applied to the transition, as well as the ground, state.

Alternatively, solvent clustering at the reacting center of the reagent and olefinic molecule in the transition state may, by simple bulk effects, discourage reaction at the sterically encumbered central portion of the system. In keeping with this explanation is the low selectivity exhibited by various "neutral" oxidizing species, *e.g.*, peracids, diimide, or osmium tetroxide,^{8,9} as contrasted with the higher selectivity of charged (and therefore more extensively solvated) agents, such as protonated N-bromosuccinimide, protons, and mercuric acetate.

Conversion of terminal bromohydrin to epoxide is readily and generally accomplished by treatment with base. Since the production of these first acyclic terpene terminal epoxides, several important natural products of this type have been identified. One of these is the famed "juvenile hormone," recently proved to be a higher homolog (19)¹¹ of the methyl farnesate case studied earlier in our laboratory. Geranylgeraniol 14,15-oxide (20), of pharmaceutical interest in that it has shown promise in the treatment of schistosomiasis, was isolated from *Pterodon pubescens*¹² after having been secured in the racemic form by synthesis at Stanford.

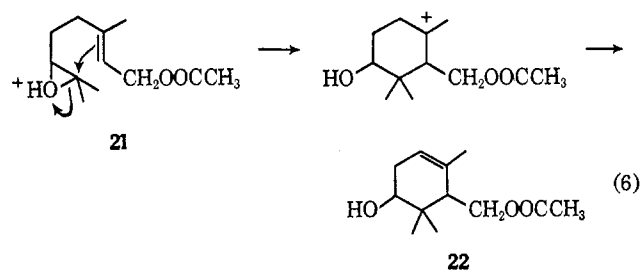


With the practical problem of selective oxidation solved, we concerned ourselves next with conversion of acyclic terpene derivatives to cyclic systems featuring the hydroxylated A-ring moiety characteristic of cyclic triterpenoid and steroid classes. It appeared that terpenoid terminal epoxides might serve as useful starting materials for cyclization reactions, and the concept was first tested with a simple model case, geranyl acetate epoxide (21) (eq 6). Ring opening

(10) E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p 151.

(11) K. H. Dahm, B. M. Trost, and H. Roller, *J. Am. Chem. Soc.*, **89**, 5292 (1967).

(12) W. B. Mors, M. F. dos Santos F.°, H. J. Monteiro, B. Gilbert, and J. Pellegrino, *Science*, **157**, 950 (1967).



accompanied by interaction with the neighboring olefinic link would be expected to involve the more stable carbonium ion centers and thus lead to a hydroxylated six-membered ring. In our 1962 experiment, the epoxide **21** was transformed by treatment with cold 85% phosphoric acid to cyclohexenol **22**.^{13,14}

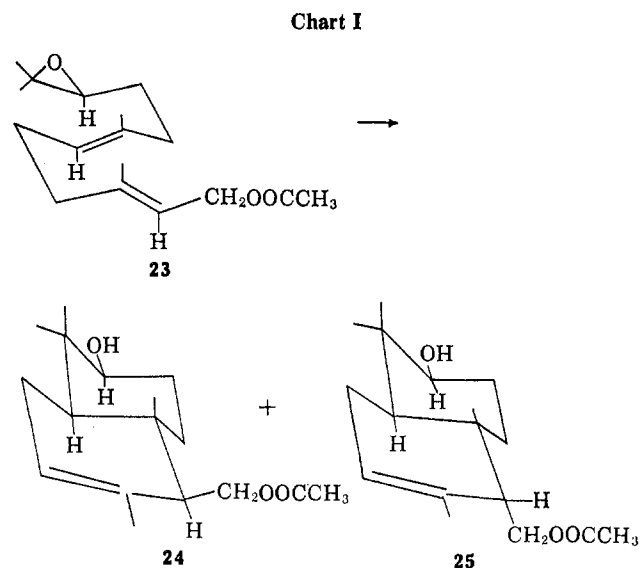
However, the challenging and significant aspect of such cyclizations was considered to be stereochemical behavior in a polycyclic context, and attention was soon turned to the more complex case of *trans,trans*-farnesyl acetate terminal epoxide (**23**). Although one can imagine various conformations which would permit epoxide ring-opening-carbocyclization reactions, the three-dimensional arrangement depicted in **23** seemed particularly attractive from the standpoint of sterol synthesis. In this conformation, the epoxide unit is so oriented that S_N2-like attack by the neighboring π electrons, coupled with epoxide ring opening, is favorable and would generate a cyclohexanol ring with the C-3 equatorial hydroxyl *trans* to the C-5 hydrogen. Assuming interaction of the developing cyclohexyl-carbonium ion with the second olefinic center—all occurring with preservation of the *trans* relationship of methyl and hydrogen on the first olefinic bond—there should evolve a *trans*-fused bicyclic carbonium ion which would solvate or deprotonate to final product.⁴

In the most stereoselective result, BF₃ etherate catalyzed in fair yield cyclization of the pure *trans,trans* isomer to a mixture of bicyclic diol monoacetates, 15% **25** and 85% **24**, the latter featuring at the four new asymmetric centers stereochemistry characteristic of the usual 3-hydroxylated A/B ring system of polycyclic terpenoids¹⁵ (Chart I). Interestingly, if the cyclization is conducted in 85% phosphoric acid, there is generated mainly the isomer (**25**) bearing a pseudo-axial acetoxymethylene substituent, of interest because (1) it features in the cyclohexene carboxylic ester ring the rare *cis* methyl-hydrogen relationship presumed to be advantageous in the methyl-hydrogen migration-elimination sequence leading in nature to lanosterol^{4a} and (2) despite the pseudo-axial substituent, but by reason of decreased interaction with both neighboring angular methyl and olefinic methyl, this isomer is probably the more stable in the **24**-**25**

(13) Unpublished results secured by Dr. A. Storni.

(14) Similar experiments with monoterpenoid epoxides have been carried out by investigators in both hemispheres (unpublished results transmitted to the author either orally or by document) including D. J. Goldsmith [*J. Am. Chem. Soc.*, **84**, 3913 (1962)], who published the 2,6-dimethylhepta-1,5-diene 5,6-oxide case.

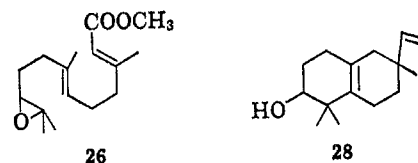
(15) E. E. van Tamen, A. Storni, E. J. Hessler, and M. Schwartz, *ibid.*, **85**, 3295 (1963).



- (a) BF₃·Et₂O-benzene on *trans,trans* acetate: 85% **24**, 15% **25**
 (b) BF₃·Et₂O-benzene on 65% *trans,trans*-35% *trans,cis* acetate: 55% **24**, 45% **25**
 (c) H₃PO₄ (85%) on *trans,trans* acetate
 (d) H₃PO₄ (85%) on 65% *trans,trans*-35% *trans,cis* acetate } 15% **24**, 85% **25**

pair. Thus the generation in nature of the B/C *syn* arrangement offers no particular obstacle, and in fact can be approximated in a laboratory cyclization. Proof of structure and stereochemistry of **24** and **25** was managed by conversion to known natural products and by LiAlH₄ reduction studies of C-3 ketone.¹⁵⁻¹⁷

Novel by-products accompanied the farnesyl acetate epoxide cyclizations, and this was true of farnesic ester epoxide (**26**) cyclizations as well. In Table II are summarized product and yield data for the latter case, which has been studied in particular detail. The bridged ether type (**27**) is formed from both the farnesyl acetate and farnesic ester epoxides; in addition, the unusual 2,2-methylvinyl system **28** arises from the farnesyl acetate epoxide cyclization.



With the basic cyclization pattern established, application of this synthetic chemistry to representative natural product cases was pursued. In the sesquiterpene category, farnesiferols A (**29**), B (**30**), and C (**31**)¹⁸ were regarded as especially suitable targets,

(16) On the basis of data cited in this 1963 paper and in later publications, supported by experiments with 6,7-*cis*-farnesic ester terminal epoxide (unpublished results secured by J. McCormick), it is clear that this stereoselective bioorganic reaction (**23** → **24** or **25**) represents an early case of formation of bicyclic product from acyclic polyene by a "nonstop" process of the biosynthetic type.⁴

(17) For an account of (a) some of the prior theoretical and experimental contributions of G. Stork, A. Eschenmoser, and others, as well as (b) his own contemporaneous activities in this area, read W. S. Johnson, *Accounts Chem. Res.*, **1**, 1 (1968).

(18) T. Caglioti, H. Naef, D. Arigoni, and O. Jeger, *Helv. Chim. Acta*, **41**, 2278 (1958); **42**, 2557 (1959).

in that the structures closely resembled those of products already secured in these studies. In the execution, terminal oxidation of umbelliferin (*trans,trans*) (32)

was realized by reaction with NBS in aqueous ethylene glycol dimethyl ether, and resulting halohydrin was converted into the *trans,trans* epoxide 33 (eq 7).

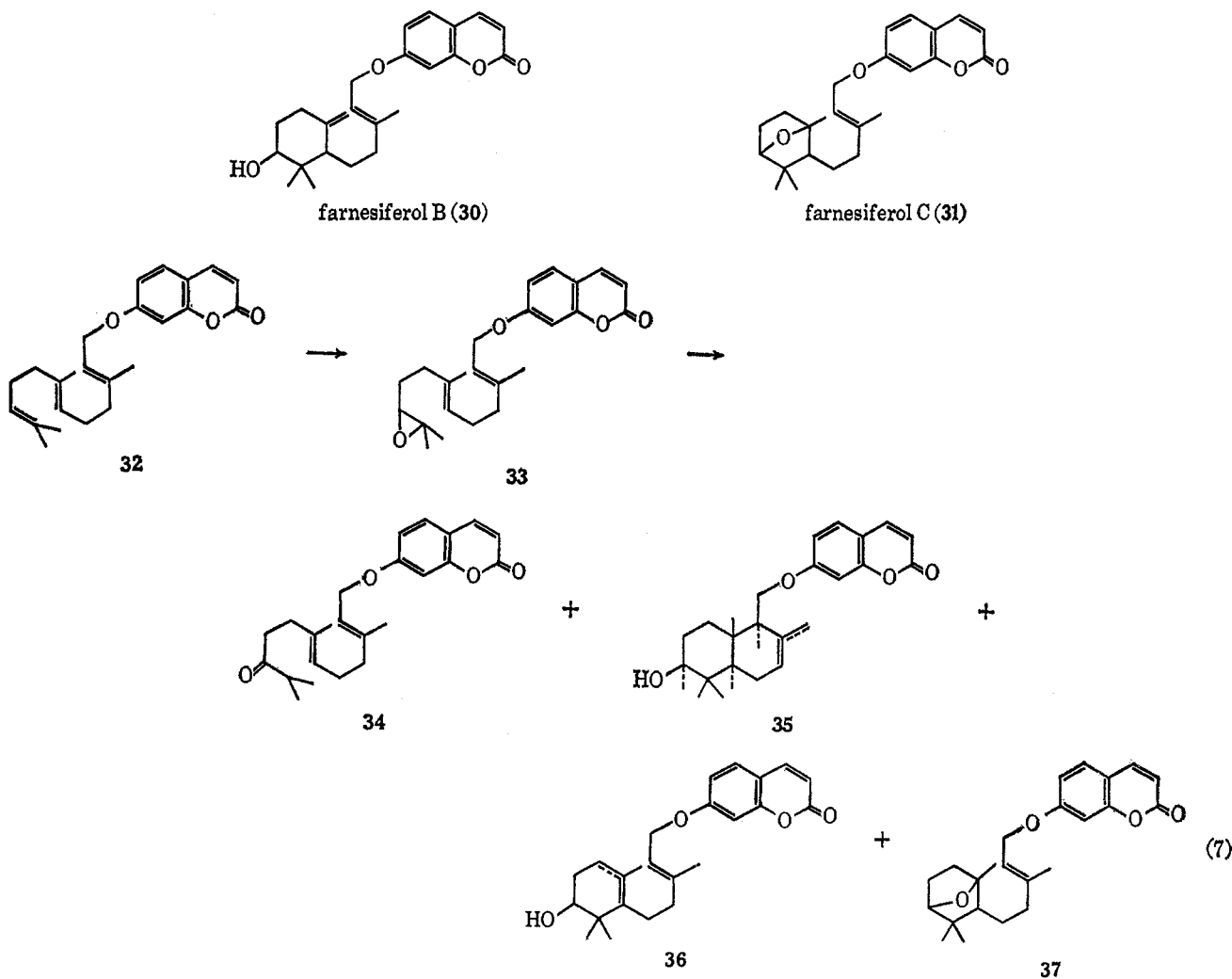
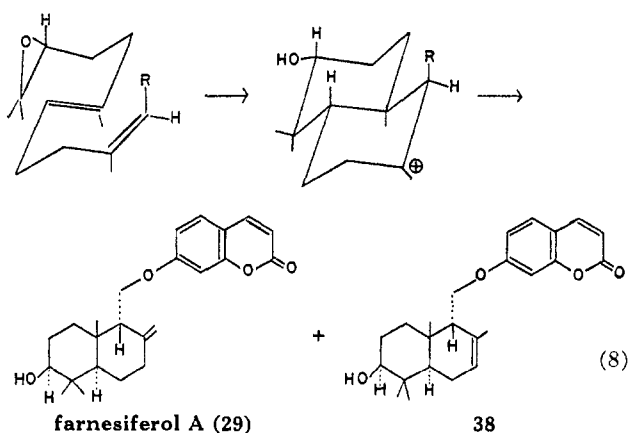


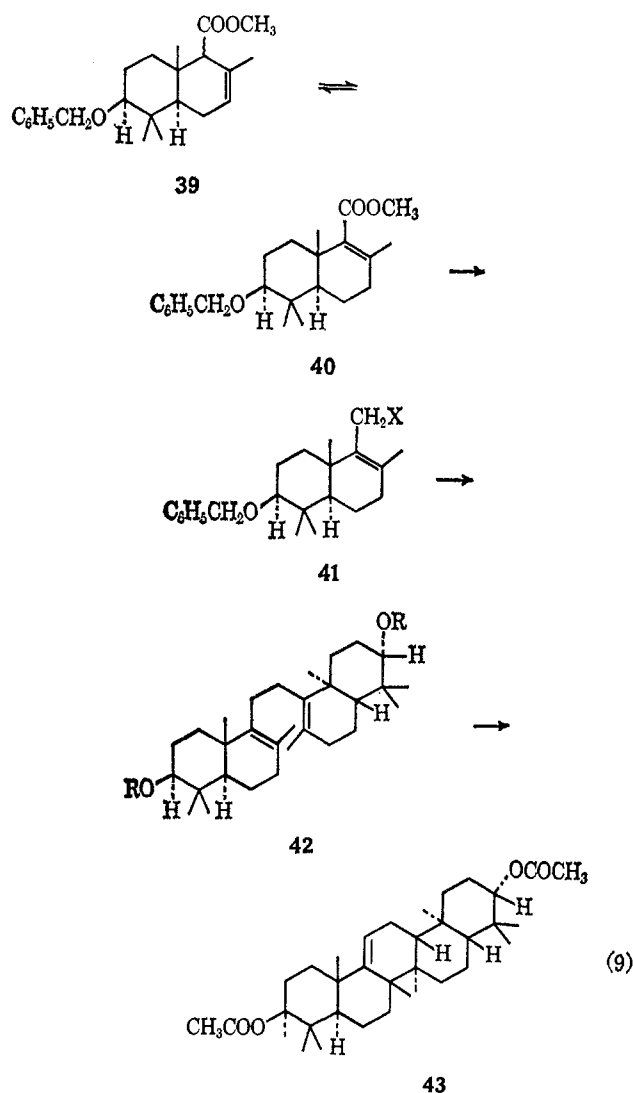
Table II
Products of Reaction of 26 (99% *trans,trans*)

Product	% yield	
	With $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (benzene)	With H_3PO_4
	92% equatorial COOCH_3 3% axial COOCH_3 5% α, β -unsatd	75% equatorial COOCH_3 15% axial COOCH_3 10% α, β -unsatd
	20%	20-30
	25%	11
	10%	None

On being subjected to the action of BF_3 etherate in benzene at room temperature, the epoxide gave rise to various products, including acyclic ketone **34**, a mixture of monocyclic (**35**) and bicyclic alcohols (**36**), and bridged ether **37**. Suitable comparison showed the synthetic bridged ether to be (\pm)-farnesiferol C. However, careful tlc inspection of synthetic bicyclic product **35** revealed that (\pm)-farnesiferol A (**29**) was *not* a component of this fraction. In order to explain this result, the assumption was made that farnesiferol A does not possess the normal *trans,anti* stereochemistry, but belonged—as tentatively suggested by Arigoni and Jeger¹⁸—to the uncommon *trans,syn* class. In an experiment designed to produce farnesiferol A (**29**) *trans,cis*-umbelliprenin terminal epoxide (**32a**) was subjected to reaction conditions approximating those described above; (\pm)-farnesiferol A (**29**) was obtained in small yield, in addition to endocyclic isomer (**38**)¹⁹ (eq 8).

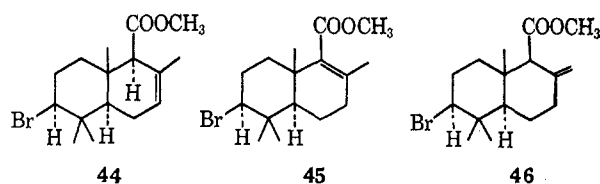


In a second application, entry into the naturally occurring pentacyclic triterpenoid area was achieved by coupling of like 3-oxygenated bicyclic sesquiterpenoid moieties to the tetracyclic onocerin system, followed by well-precedented acid-catalyzed cyclization to the pentacyclic level.²⁰ Coupling was effected by treatment with magnesium of the allylic bromide **41** ($\text{X} = \text{Br}$), obtained as the result of a sequence (eq 9) involving isomerization of O-benzyl ether **39** to α,β -unsaturated ester **40**, resolution, reduction to allyl alcohol **41** ($\text{X} = \text{OH}$), and treatment with HBr . After reductive cleavage and acetylation, the bis ether **42** ($\text{R} = \text{C}_6\text{H}_5\text{CH}_2$) gave rise to diacetate identical in all respects with (+)- β -onocerin diacetate (**42**, $\text{R} = \text{CH}_3\text{CO}$). As would be anticipated on the basis of earlier observations, the onocerin diacetate provided, on treatment with acetic-sulfuric acids, a modest yield of pentacyclic product, the γ -onocerin diacetate **43**.²¹ In view of the recorded conversion of this pentacycle to hopenone-I, the over-all synthetic operation constitutes a total



synthesis of the naturally occurring pentacyclic triterpenoid system.²²

It is noteworthy that small yields of bromo bicyclic esters (**44–46**) are formed *directly* during the NBS bromination of methyl farnesate. Investigations described elsewhere suggest that these substances do not derive from bromohydrin or monocyclic intermediate,



but emerge by a "nonstop" cyclization induced by attack of oxidizing agent on acyclic material.²³

Can the epoxide cyclization method be extended to

(19) E. E. van Tamelen and R. M. Coates, *Chem. Commun.*, 413 (1966).

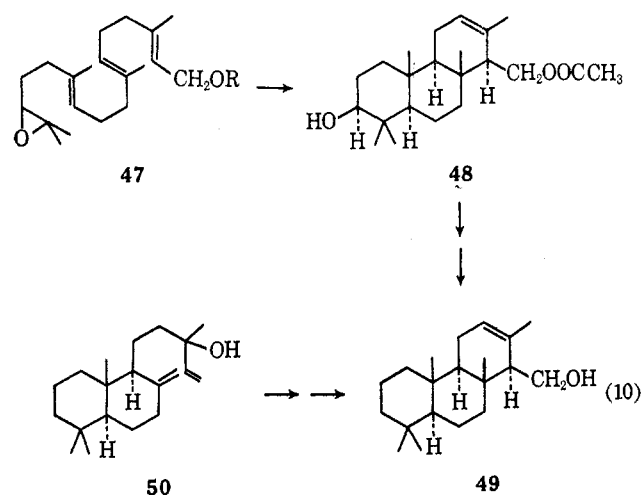
(20) E. E. van Tamelen, M. A. Schwartz, E. J. Hessler, and A. Storni, *ibid.*, 409 (1966).

(21) D. H. R. Barton and K. H. Overton, *J. Chem. Soc.*, 2639 (1955).

(22) For the previous synthesis of α -onocerin and hopenone-I, see G. Stork, J. E. Davies, and A. Meisels, *J. Am. Chem. Soc.*, **81**, 5516 (1959); **85**, 3419 (1963).

(23) E. E. van Tamelen and E. J. Hessler, *Chem. Commun.*, 411 (1966).

tricyclic cases? In an endeavor to answer this question, the oxidation-cyclization sequence was applied to *trans,trans,trans*-geranylgeraniol,²⁴ made available by homologation of *trans,trans*-farnesol. Oxidation of the diterpenoid O-acetate by NBS followed by treatment with base provided epoxide (**47**, R = H), which as the O-acetate (**47**, R = CH₃CO) was cyclized by exposure to 0.2 molar equiv of SnCl₄ in benzene at 0°. After routine work-up and chromatography, various entities were isolated, including a certain amount of tricyclic diol monoacetate **48** (eq 10). Reliable chemical evi-

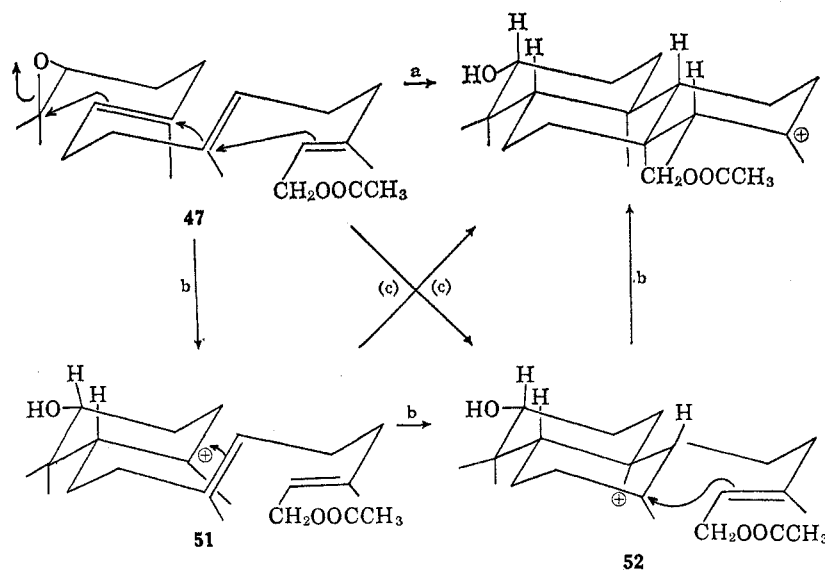


at three centers with chemically preferred, *t*-carbocationic properties.

In the sesquiterpene series, we have shown that monocyclic products obtained by cyclization of terminal epoxide are not separately converted under original cyclization conditions to bicyclic product formed concurrently from the epoxide, and therefore are not intermediates in the formation of the latter.²³ On the basis of this analogy, we believe (Chart II) that the tricyclic diol monoacetate **48** arises from epoxide **47** by (a) a synchronized cyclization, (b) a stepwise sequence involving mono- (**51**) and bicyclic (**52**) carbonium ion, or (c) a sequential combination of mechanisms a and b. In any case, from epoxide **47** there is built up in one laboratory operation a 3-hydroxylated tricyclic terpene featuring *six* asymmetric centers specifically oriented in the relationship most common in the natural product series.

In view of the foregoing oxidation and cyclization results we began to consider whether the over-all bio-conversion of squalene to sterols might in fact proceed by way of squalene 2,3-oxide as a genuine intermediate. Availability, by virtue of our NBS oxidation method, of the required radio-labeled epoxide provided the opportunity to test this hypothesis, and, in collaboration with Professor R. B. Clayton of Stanford University, experiments along these lines were started in

Chart II



dence for the molecular and stereochemical nature of the tricyclic substance was obtained by conversion (Raney nickel hydrogenolysis of dithioacetal derived from C-3 ketone) to a transformation product **49** also secured in a rational manner from the naturally occurring manool (**50**). Thus the head-to-tail arrangement of isoprenoid units in this case allows formation of a hydrophenanthrene system through bond formation

autumn 1965. In anaerobic experiments with rat liver enzyme preparations carried out during the first half of 1966, we found that ¹⁴C-labeled lanosterol was efficiently synthesized from ¹⁴C-labeled squalene 2,3-oxide, and that cholesterol was produced under aerobic, but otherwise similar, conditions.²⁵⁻²⁷ These results were fortified by the observation that in aerobic sys-

(24) E. E. van Tamelen and R. G. Nadeau, *J. Am. Chem. Soc.*, **89**, 176 (1967).

(25) E. E. van Tamelen, J. D. Willett, R. B. Clayton, and K. E. Lord, *ibid.*, **88**, 4752 (1966).

(26) J. D. Willett, K. B. Sharpless, K. E. Lord, E. E. van Tamelen, and R. B. Clayton, *J. Biol. Chem.*, **242**, 4182 (1967).

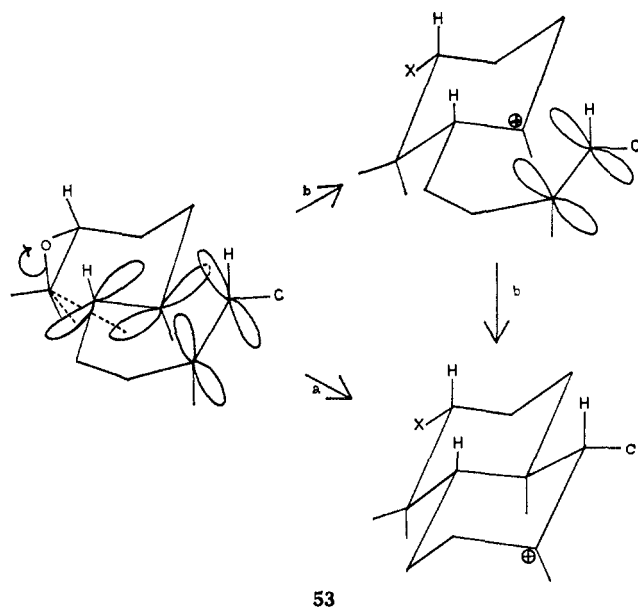
tems ^{14}C -squalene generated isolable amounts of labeled squalene 2,3-oxide.²⁵⁻²⁷ Finally, the central role of squalene oxide in sterol synthesis was established by (1) the retention of both atomic labels in the conversion of ^3H , ^{18}O -labeled substrate to lanosterol, (2) lack of observable conversion to sterol of ^3H -labeled 2,3-dihydrosqualene-2,3-diol, 2,3-dihydrosqualen-2-ol, and squalene 2,3:22,23-dioxide, and (3) apparent absence of any cofactor requirements for the microsomal enzyme system utilized in the cyclization.^{26,28,29}

Accepting squalene oxide as a true biological intermediate, we feel justified in applying our knowledge of the organic chemistry of terpene epoxides in interpretations of the enzymic process, especially since parallelism between the biochemical and nonbiochemical modes is apparent in no less than three behavioral facets: (1) selective terminal oxidation of acyclic terpenes as a distinct, separate step, (2) cyclization of terpene oxides to 3-hydroxylated polycyclic systems possessing the "natural" stereochemistry, and (3) annulation by mechanistic means which apparently do not involve partially cyclized intermediates which are reprotated *en route*.

In regard to the oxidation step, the high selectivity attainable in nonenzymic reactions suggests that the purely physicochemical factors available may lower the entropy demands on "squalene epoxidase" and reduce the energy of the transition state. As a consequence, a corresponding limitation on the structural specificity of the enzyme would be anticipated, thereby permitting oxidation of olefins other than squalene.

Insofar as the cyclization of a terminal epoxide is concerned, the π -electron orbitals of the closest olefinic bond are ideally directed in space for interaction with a developing empty orbital at the more highly substituted position of the epoxide unit, implying that a combined oxide ring opening-carbocyclization process having $\text{S}_{\text{N}}2$ character is easily possible and may well be anchimerically assisted (**53**). As far as the several successive π bonds *per se* in terpenes are concerned, the orbital geometry and symmetry characteristics are not conducive to interaction leading to cyclization. However, with positive charge developing at the more highly substituted carbons during acid-promoted epoxide ring opening, interaction with neighboring π electrons becomes increasingly felicitous, the carbon framework maintaining all the while the approximate geometry and conformation required for ultimate formation of the polycyclic system with natural stereochemistry. The extent of positive charge localization or delocalization remains unknown; more information is needed for locating the chemical process in the continuum bounded

by a completely synchronized operation on the one hand (a), and a sequence featuring discrete molecular species with carbocationic centers on the other (b).



Applying the above considerations specifically to the lanosterol biosynthesis picture previously developed by Eschenmoser and Stork,⁴ we find ourselves with the mechanistic view shown as **54**. In this interpretation, as before, elaboration of a six-membered C ring poses, from the organic chemical standpoint, a problem, in that utilization of the more stable tertiary carbocationic center would result instead in formation of a five-membered C ring. This simple prediction was borne out in the nonenzymic cyclization of squalene oxide, which produced two identified tricyclic products, (1) the trienic alcohol **57** with the squalene skeleton intact, and (2) a rearranged isomer (**58**).³⁰ The problem can be avoided by invoking enzymatic intervention or by resorting to an alternate biochemical mechanism (**55** \rightarrow **56** \rightarrow lanosterol) which, still consistent with all available tracer experiments, assigns the role of actual intermediate to a perhydrocyclopentanophthalene.²⁸

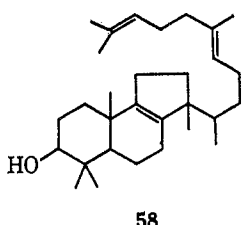
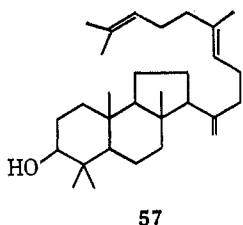
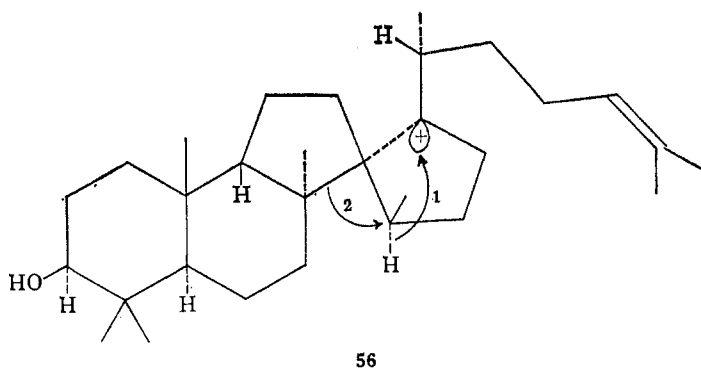
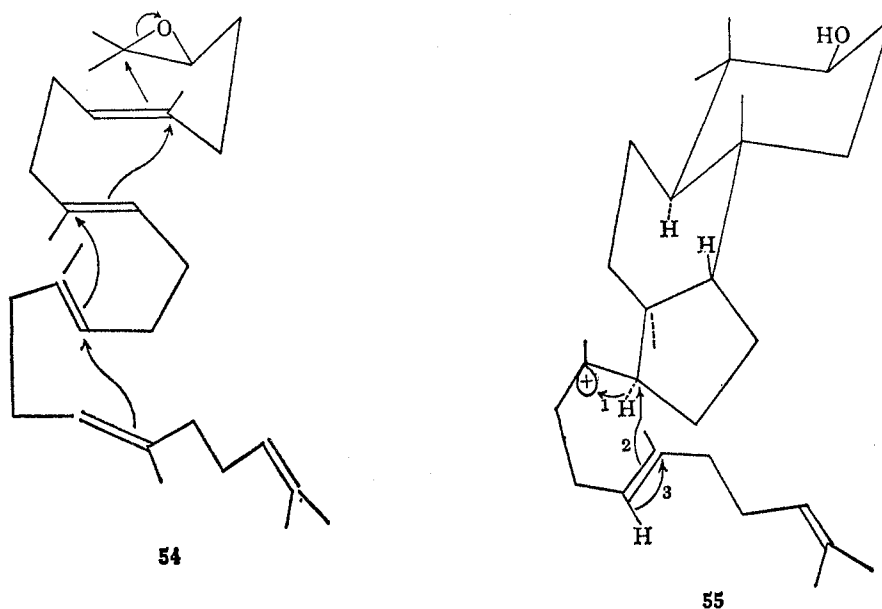
Because of the foregoing considerations, further investigation of the biological cyclization mechanism was called for. Furthermore, means for execution of revealing experiments were now available, in that the biochemical investigations outlined above had demonstrated for the first time that the over-all squalene to lanosterol conversion consisted of two main, separate steps, each amenable to study in its own right. One approach which promised good return on investment of scientific effort was administration of structurally modified squalene oxides to the *in vitro* enzyme system, and structural study of any biogenic products. We

(27) E. J. Corey and W. E. Russey [*J. Am. Chem. Soc.*, **88**, 4750, 4751 (1966)], using squalene 2,3-oxide prepared by means of our selective NBS oxidation method,¹¹ provided welcome independent confirmation of these results. We are grateful to Dr. Russey for informing us of his intentions along these lines while our own enzymic experiments were in progress.

(28) E. E. van Tamelen, J. D. Willett, and R. B. Clayton, *ibid.*, **89**, 3371 (1967).

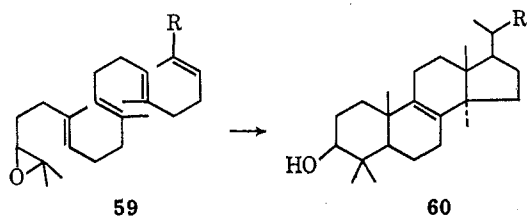
(29) For an authoritative review of earlier sterol biosynthesis research, see R. B. Clayton, *Quart. Rev. (London)*, **19**, 168 (1965).

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



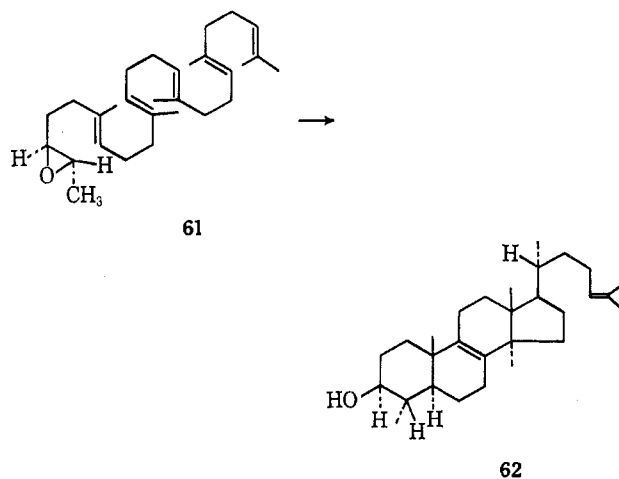
have now embarked upon, with Professor Clayton, a broad program which involves the specific synthesis and enzymic feeding of radioactive squalene oxide modifications in which the (1) olefinic terminus, (2) epoxide moiety, or (3) central portion of the potential substrate is varied.

Our first experiments along these lines were concerned with type (1), and in these endeavors we observed the first conversion of squalene and squalene oxide variants (59) to modified sterols (60): 2,3-dihydro- and 1,1',2-



trisnorsqualene 22,23-oxides were transformed into 24,25-dihydro- [60, R = (CH₂)₃CH(CH₃)₂] and 25,26,27-trisnorlanosterols [60, R = (CH₂)₂CH₃], respectively.³¹ Even the sesterterpene oxide (59, R = CH₃) leads to sterol-like product.³²

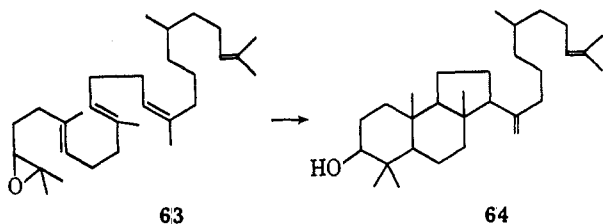
Turning to the other extremity of the squalene oxide system, we learned that the absence of a particular methyl group from the epoxide ring can be tolerated: the trans epoxide 61 is enzymically transformed to



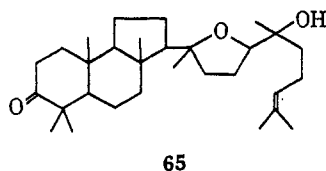
(31) E. E. van Tamelen, K. B. Sharpless, J. D. Willett, R. B. Clayton, and A. L. Burlingame, *J. Am. Chem. Soc.*, **89**, 3920 (1967).
 (32) Results secured at Stanford University by Mr. R. Hanzlik.

4 α ,14 α -dimethyl- $\Delta^{8,24}$ -cholestadienol (**62**). However, neither the *cis* isomer of **61** nor the 1,1'-bisnor epoxide is detectably converted to sterol.³³

Although possible variations within the interior of the squalene structure are numerous, we report at this time the results of only one significant case. As indicated by comparison with products of nonenzymic cyclizations, *trans,trans,trans*-18,19-dihydrosqualene 2,-



3-oxide (**63**) yields with the rat liver enzyme preparation a perhydrocyclopentano[*a*]naphthalene (**64**).³⁴ Thus validation of the predicted biological conversion of a squalene oxide type to a tricyclic species with a five-membered C ring is provided by this case, as well as by the identification of a plant product (**65**) as a member of the same class.³⁵



Our present and future activities concern both organic and biochemical problems. In the former category falls the biogenetic-type synthesis of lanosterol itself from an appropriate epoxide precursor. Also, a fuller understanding of the purely physical organic aspects of epoxide formation and cyclization is deemed necessary for better appreciation of the behavior of squalene oxide in biochemical processes. On the more biological side, our present efforts are concerned to a large degree with gathering evidence that will permit tracing the exact course of squalene oxide in its journey to lanosterol, including the detailed stereochemical aspects of such movements. A beginning has been made in the trying task of solubilizing and purifying the cyclization enzyme(s), in preparation for later structure determination and identification of active sites in these materials. Enlightened by results of the above programs, we hope to reach the ultimate goal: a detailed, cinematographic-like view of how enzyme molecules

act on squalene and its oxide, inducing conversion to sterols.

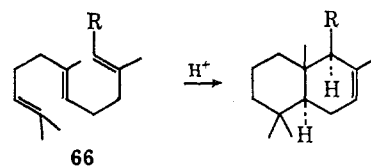
Appendix

Having presented a chemical narrative of work based on "biogenetic-type synthesis," we comment on some aspects of terminology. Since the definition and suggested usage of this term in 1961,³⁶ it has been employed to describe a wealthy spectrum of biochemically based reactions executed in the organic laboratory without the agency of enzyme catalysts. Concurrently, the need for additional definition and classification within this context has become apparent, and we propose subdivision of "biogenetic-type reactions" into three categories.

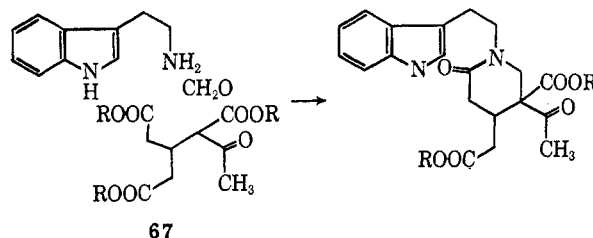
Type A. Amphosynthesis. A clearly defined, spontaneous nonenzymic reaction of a given molecular entity which proceeds by exactly the same mechanistic path in nature as in the organic laboratory. This organic-biochemical borderline type is likely to be rare, but may include, for example, certain reactions of phenoxy radicals. This type of reaction will invariably lead to a racemic product when an optically inactive molecule serves as starting material.

Type B. Bioorganic Synthesis. An organic, nonenzymic transformation which is carried out on a molecule of precisely the same chemical type as the natural substrate and which leads to product similar to, if not identical with, material produced by the corresponding enzymic change. As elaborated above, the conversion of acyclic terpene terminal epoxides to 3-hydroxylated polycyclic systems conforms to this pattern.

Type C. Chemical Analog of a Biosynthesis. A purely organic synthetic reaction which is roughly patterned after a biological prototype, but is recognized as distinctly different in more than trivial detail. In this type, a natural substrate type is not used, but instead starting material only approximately similar to biosynthetic substrate. Although not a close simulation of the biochemical change, the realization of the analogy in the nonenzymic laboratory can have important thermodynamic, stereochemical, and/or practical implications. As an example, the nonoxidative cyclization parallel (**66**) for sterol formation from squalene may be cited.⁴ Also, the condensation-cyclization **67**



serves as a biogenetically patterned device for efficient assemblage



of an indole alkaloidal "skeleton," without its being molecularly identical with the enzymic construction.³⁷

(33) R. B. Clayton, E. E. van Tamelen, and R. G. Nadeau, *J. Am. Chem. Soc.*, **90**, 820 (1968).

(34) E. E. van Tamelen, K. B. Sharpless, R. Hanzlik, R. B. Clayton, A. L. Burlingame, and P. Wszolek, *ibid.*, **89**, 7150 (1967).

(35) A. Chawla and S. Dev, *Tetrahedron Letters*, 4837 (1967).

(36) E. E. van Tamelen, *Fortschr. Chem. Org. Naturstoffe*, **19**, 245 (1961).

(37) E. E. van Tamelen and C. Placeway, *J. Am. Chem. Soc.*, **83**, 2594 (1961).