Some physical properties of pentaprismane are listed in Table I, along with those of triprismane (Ladenburg's benzene)[14] and tetraprismane (cubane),[15] the only other known parent prismanes. We shall report on the chemistry of pentaprismane as soon as possible.

Acknowledgment. The research effects of the principal investigator were supported by the National Science Foundation (CHE-78-07430) and the National Cancer Institute of the National Institutes of Health (CA-12961). The NMR instruments used were funded in part by the NSF Chemical Instrumentation Program and by NCI via the University of Chicago Cancer Research Center (CA-14599).

(14) (a) Katz, T. J.; Acton, N. *J. Am. Chem. Soc.* **1973**, *95*, 2738. (b) Turro, N. J.; Ramamurthy, V.; Katz, T. J. *Nouv. J. Chim.* **1977**, *1*, 363.
(15) From Table I: Eaton, P. E. *Tetrahedron* **1979**, *35*, 2189.

Trichodiene Biosynthesis and the Enzymatic Cyclization of Farnesyl Pyrophosphate

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The original formulation of the Isoprene Rule, and its evolution into the Biogenetic Isoprene Rule, remains one of the major theoretical achievements of modern organic chemistry.[1] On the basis of the unifying concept that a single acyclic activated substrate, farnesyl pyrophosphate (**1**), serves as the precursor of all sesquiterpenes, extensive biogenetic schemes have been proposed by several authors to account for the formation of the nearly 200 different carbon skeletons in this family of natural products.[2] In spite of these theoretical achievements, however, the precise stereochemistry of the farnesyl pyrophosphate isomer which undergoes cyclization has been a subject of considerable debate.[3] The difficulty stems from the realization that the formation of six-membered rings from trans-allylic pyrophosphates requires isomerization of the trans-2,3 double bond in order to avoid formation of *trans*-cyclohexene (Scheme IA). Most available evidence is consistent with initial formation of *trans,trans*-farnesyl pyrophosphate, and attempts to explain the subsequent isomerization and cyclization have generated two conflicting theories: (1) Trans-cis isomerization-cyclization via the intermediate tertiary allylic pyrophosphate, nerolidyl pyrophosphate (**2**), has been suggested by several groups of investigators[3,4] (Scheme IA). (2) Various redox schemes for the trans-cis isomerization have been proposed.[3] Although differing in detail, these latter theories all require removal of one of the C-1 hydrogen atoms. For example, several authors have proposed that isomerization might occur at the level of the derived unsaturated aldehyde, farnesal (**3**) (Scheme IB). Much of the evidence bearing on both theories has recently been reviewed in detail[3] and need not be considered here. One of the strongest arguments favoring the redox theories, however, has been the claim by Hanson[5] that conversion of farnesyl pyrophosphate to trichodiene (**5**), the parent hydrocarbon of the trichothecane family of sesquiterpene antibiotics,[6,7] involves loss of a C-1 hydrogen atom. Thus incubation of [1,5,9-$^3H_6$,4,8,12-$^{14}C_3$]-*trans,trans*-farnesyl pyrophosphate [$^3H/^{14}C$ atom ratio 6:3] with a cell-free system from *Trichothecium roseum* was reported to yield trichodiene [$^3H/^{14}C$ atom ratio 5.2:3]. This original report did not verify the reported isotope ratios of either substrate or product by recrystallization of suitable derivatives to constant activity or substantiate the apparent isotope distribution by the requisite chemical degradations.[8] Several recent studies have cast doubt on the generality of the Sussex group's findings. In a series of careful investigations using enzymes from sage and fennel, Croteau has demonstrated that several cyclic monoterpenes are biosynthesized without loss of isotope from C-1 of geranyl pyrophosphate and with no requirement for nicotinamide coenzymes.[11] Independently Arigoni and Gotfredsen have found that the whole cell biosynthesis of the sesquiterpene coccinol (**6**), a metabolite of *Fusidium coccineum*, takes place with complete retention of tritium from [5-$^3H_2$,2-^{14}C]mevalonate.[12] The latter result is particularly relevant since coccinol is apparently derived from a bisabolyl cation (**4**) similar to that which ultimately gives rise to trichodiene. We have therefore reexamined the cell-free biosynthesis of trichodiene and our results, reported below, establish unambiguously that conversion of *trans,trans-farnesyl pyrophosphate to trichodiene takes place without loss of hydrogen from C-1 of the precursor.*

trans,trans-[1-^3H,12,13-^{14}C]Farnesol was synthesized as previously described[13] and a portion was converted to farnesyl diphenyl

Table I. Conversion of [1-$^3H_2$,12,13-^{14}C]Farnesyl Pyrophosphate to Trichodiene and Distribution of the Label

compd	^{14}C specific activity, dpm/mmol	$^3H/^{14}C$
1[a]	1×10^8	9.04 ± 0.12[b]
5[c]	5.06×10^4 [d]	8.70
8	5.32×10^4	9.29
11	6.14×10^4	8.23 ± 0.12
13	6.23×10^4	0.0
15	5.84×10^4	4.49 ± 0.09

[a] Amount incubated, 1×10^6 dpm ^{14}C (10 μmol). [b] Based on recrystallization of farnesyl diphenylurethane. [c] Total recovered activity, 1.8×10^4 dpm ^{14}C. [d] Diluted to 110 mg.

† Fellow of the Alfred P. Sloan Foundation, 1978-1982; National Institutes of Health Research Career Development Award, 1978-1983.

(1) L. Ruzicka, *Pure Appl. Chem.*, **6**, 493 (1963); *Proc. Chem. Soc.*, 341 (1959).
(2) J. B. Hendrickson, *Tetrahedron*, **7**, 82 (1959); J. H. Richards and J. B. Hendrickson, "The Biosynthesis of Steroids, Terpenes, and Acetogenins", W. A. Benjamin, New York, 1964, pp 225-239; W. Parker, J. S. Roberts, and R. Ramage, *Q. Rev. Chem. Soc.*, **21**, 331 (1967); T. K. Devon and A. I. Scott, "Handbook of Naturally Occurring Compounds", Vol. 2, Academic Press, New York, 1972, pp 56-57.
(3) For a recent review, see D. Cane, *Tetrahedron*, **36**, 1109 (1980).
(4) See D. Arigoni, *Pure Appl. Chem.*, **41**, 219 (1975); N. H. Andersen, Y. Ohta, and D. D. Syrdal In "Bio-organic Chemistry", Vol. 2, E. E. van Tamelen, Ed., Academic Press, New York, 1977, pp 1-37; N. H. Andersen and D. D. Syrdal, *Tetrahedron Lett.*, 2455 (1972); D. V. Banthorpe, B. V. Charlwood, and M. J. O. Francis, *Chem. Rev.*, **72**, 115 (1972); S. Gotfredsen, J. P. Obrecht, and D. Arigoni, *Chimia*, **31** (2), 62 (1977).
(5) R. Evans, A. M. Holton, and J. R. Hanson, *J. Chem. Soc., Chem. Commun.*, 465 (1973); R. Evans and J. R. Hanson, *ibid.*, 231 (1975); *J. Chem. Soc., Perkin Trans. 1*, 326 (1976).
(6) S. Nozoe and Y. Machida, *Tetrahedron*, **28**, 5105 (1972); Y. Machida and S. Nozoe, *Tetrahedron Lett.*, 1969 (1972).
(7) For recent reviews, see (a) Ch. Tamm and W. Breitenstein in "The Biosynthesis of Mycotoxins", P. S. Steyn, Ed., Academic Press, New York, 1980, pp 69-104; (b) D. E. Cane in "Biosynthesis of Isoprenoid Compounds", J. W. Porter, Ed., Wiley, New York, 1981; (c) G. A. Cordell, *Chem. Rev.*, **76**, 425 (1976).
(8) This report also contained the implausible suggestion that farnesyl pyrophosphate isomerization might take place via a cyclopropene intermediate, thereby requiring that NADPH function as a proton rather than a hydride donor. This error continues to find its way into the review literature.[7a,9] Most recently Banthorpe[10] has chosen to challenge this dubious hypothesis, not on fundamental mechanistic grounds, but on the erroneous inference that the intermediacy of a cyclopropyl cation would necessitate the exchange of C-1 and C-2 of farnesyl pyrophosphate. Not only is the latter premise conceptually incorrect, it is also contradicted by a large body of experimental data, including that for the biosynthesis of the trichothecanes themselves![7]
(9) D. V. Banthorpe and B. V. Charlwood, *Terpenoids Steroids*, **7**, 194 (1977).
(10) A. Akhila and D. V. Banthorpe, *Phytochemistry*, **19**, 1691 (1980).
(11) (a) R. Croteau and M. Felton, *Arch. Biochem. Biophys.*, in press; (b) R. Croteau, M. Felton, and R. C. Ronald, *ibid.*, **200**, 524, 534 (1980); (c) R. Croteau and F. Karp, *ibid.*, **198**, 512 (1979); (d) R. Croteau and F. Karp, *ibid.*, **184**, 77 (1977); (e) R. Croteau in "Biosynthesis of Isoprenoid Compounds", J. W. Porter, Ed., Wiley, New York, 1981. See also T. Suga, T. Shishibori, and H. Morinaka, *J. Chem. Soc., Chem. Commun.*, 167 (1980).
(12) (a) S. E. Gotfredsen, Dissertation ETH Zurich No. 6243, 1978; (b) described in ref 3, pp 1150-1151 and in ref 7b.

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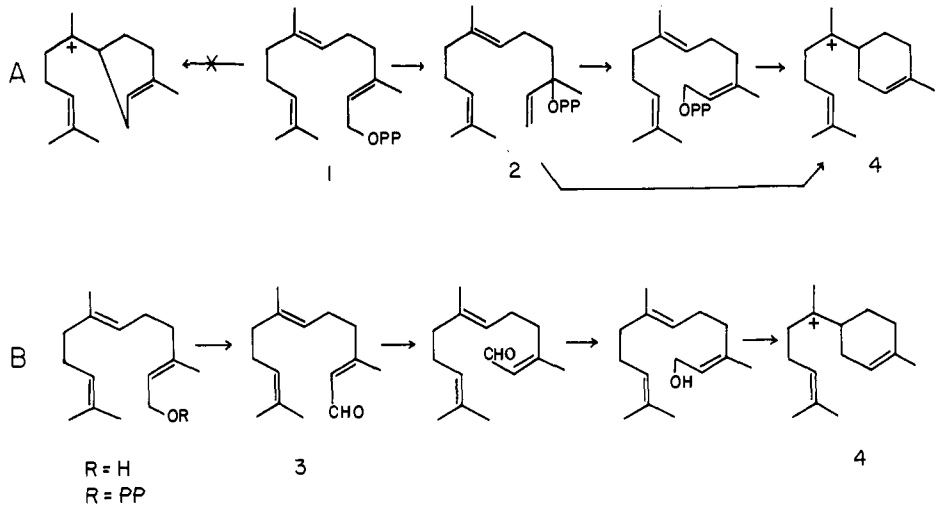
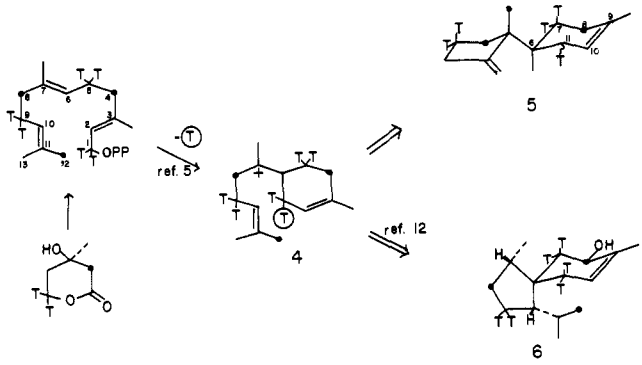
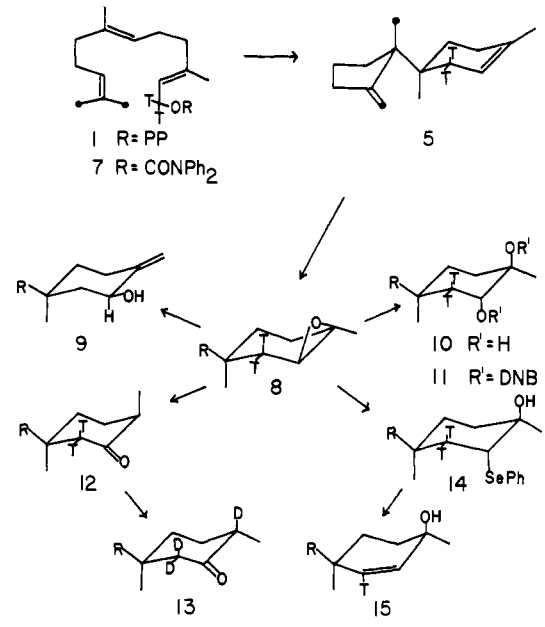
Scheme I



Scheme II



Scheme III



urethane (**7**)[14], which was recrystallized to constant activity ($^3H/^{14}C$ = 9.04 ± 0.12; atom ratio 2:2) (Table I). The remaining alcohol was then used to prepare the corresponding pyrophosphate by standard methods.[13,15]

A cell-free extract was prepared by suspending the mycelium from 500 mL of a 4-day-old culture of *Trichothecium roseum* ATCC 8685 in 30 mL of 0.1 M potassium phosphate buffer, pH 7.0, and passing the suspension through a prechilled French press at 18 000 psi and 4 °C. The extract was separated from broken cell debris by centrifugation at 4 °C, first at 10000g for 20 min and then at 17000g for 90 min. The resultant supernatant (~10 mg protein/mL)[16] was used immediately. Initial small-scale incubations established that neither the efficiency of conversion of farnesyl pyrophosphate to trichodiene nor the resultant $^3H/^{14}C$ ratio of the isolated trichodiene were affected by the presence of a mixture of NAD^+, NADH, and NADPH, similar to that used in the original study.[5,17] Nicotinamide coenzymes were therefore omitted during subsequent incubations.

For the preparative scale experiment the incubation mixture contained 1600 mg of S_{17} protein (obtained from 2.4 L of *T. roseum* culture), 2 mmol of $MgCl_2$, 0.08 mmol of $MnCl_2$, 0.1 mmol of dithiothreitol, 4.0 mmol of potassium phosphate buffer, pH 7.0, and 10 μmol of [1-^{3}H,12,13-^{14}C]farnesyl pyrophosphate in a total volume of 220 mL. After 2 h at 30 °C the reaction was quenched by addition of 12 N sodium hydroxide (25 mL) and 95% ethanol (25 mL), allowed to stand for 30 min, and then extracted with 120 mL of *n*-pentane containing 5 mg of carrier trichodiene. After purification by preparative TLC (silica gel, hexane), the recovered trichodiene ^{14}C activity (1.8×10^4 dpm) corresponded to the formation of 0.05 nmol of trichodiene/mg protein per hour. The reisolated trichodiene was mixed with an additional 105 mg of trichodiene and converted to the previously described 9,10-epoxytrichodiene (**8**),[6] by reaction with 1.05 equiv of *m*-chloroperbenzoic acid in methylene chloride.[18] A portion of the epoxide **8** was converted first to the 9,10-diol (**10**), (3%

(13) D. E. Cane, R. Iyengar, and M.-S. Shiao, *J. Am. Chem. Soc.*, **103**, 914 (1981); D. E. Cane and R. Iyengar, *ibid.*, **101**, 3385 (1979); D. E. Cane, R. Iyengar, and M.-S. Shiao, *ibid.*, **100**, 7122 (1978); D. E. Cane and R. Iyengar, *ibid.*, **100**, 3256 (1978).

(14) B. B. Bates, D. M. Gale, and B. J. Gruner, *J. Org. Chem.*, **28**, 1086 (1962).

(15) G. Popjak, J. W. Cornforth, R. H. Cornforth, R. Ryhage, and D. S. Goodman, *J. Biol. Chem.*, **237**, 56 (1962); F. Cramer and W. Boehm, *Angew. Chem.*, **71**, 755 (1959); A. A. Kandutsch, H. Paulus, E. Levin, and K. Bloch, *J. Biol. Chem.*, **239**, 2507 (1964); P. W. Holloway and G. Popjak, *Biochem. J.*, **104**, 57 (1967); W. W. Epstein and H. C. Rilling, *J. Biol. Chem.*, **245**, 4597 (1970).

(16) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

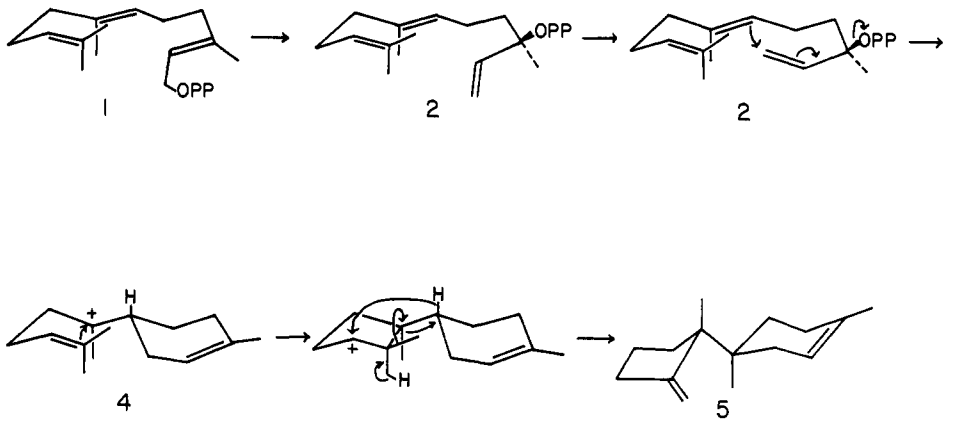
(17) Reference 5 reported that use of [4-^{3}H]NADPH gave trichodiene which was said to bear tritium, without verification by appropriate recrystallizations or degradations.

(18) Rearrangement of unlabeled **8** with diethylaluminum tetramethylpiperidide[19] gave the previously described[6] allylic alcohol **9**. In the 270-MHz ^1H NMR spectrum of **9**, the H-10 carbinyl proton was coupled to the H-11 protons with J = 4.9 and 11.5 Hz, characteristic of an axial proton. Assuming that the bulky 1-methyl-2-methylenecyclopentyl substituent must be equatorial, **8** therefore corresponds to the β-epoxide. The observed epoxidation stereochemistry was anticipated based on the results of Rickborn's earlier study of the epoxidation of 4-substituted cyclohexenes.[20]

(19) A. Yasuda, S. Tanaka, K. Oshima, H. Yamamoto and H. Nozaki, *J. Am. Chem. Soc.*, **96**, 6513 (1974).

(20) B. Rickborn and S.-Y. Lwo, *J. Org. Chem.*, **30**, 2212 (1965).

Scheme IV



$HClO_4$, THF:H_2O, 1:1/48 h/0 °C) and thence to the corresponding 9,10-bis(dinitrobenzoate) (**11**) which was recrystallized to constant activity and isotope ratio ($^3H/^{14}C = 8.23 \pm 0.12$; atom ratio 1.8:2), mp 220 °C dec. Rearrangement of a further portion of the epoxide with lithium perchlorate in benzene[21] (4 equiv/48 h/reflux) gave the 10-ketone (**12**) which epimerized to the more stable equatorial isomer **13** and lost all tritium activity (>99%) upon exchange with sodium deuteroxide in refluxing dioxane–D_2O for 30 h. The latter experiment thus located all the tritium isotope at the expected site,[7] C-11, of trichodiene.[22] In further confirmation of the above results, the corresponding 9-hydroxy-10-phenylselenide (**14**), obtained by reaction of the remaining epoxide with sodium phenylselenide (EtOH/20 h/reflux), lost one-half the total tritium activity upon treatment with sodium periodate (H_2O/THF/MeOH/0 °C/3 h and then reflux/4 h) and elimination of the selenoxide to yield the allylic alcohol (**15**).[23]

The above described experiments (Scheme III) conclusively demonstrate that the enzymatic cyclization of *trans,trans*-farnesyl pyrophosphate to trichodiene takes place without loss of isotope from C-1 of the precursor. This result, together with the previously cited findings of both Croteau and Arigoni, as well as related studies by Arigoni on the biosynthesis of a group of closely related cadalane- and humulane-derived sesquiterpenes[24] clearly exclude all redox mechanisms proposed to date and strongly favor an alternative isomerization–cyclization mechanism involving the tertiary allylic isomer nerolidyl pyrophosphate.[25] As illustrated in Scheme IV, isomerization of *trans,trans*-farnesyl pyrophosphate will give nerolidyl pyrophosphate, a conversion which we have previously shown takes place with syn stereochemistry via the allylic cation–pyrophosphate anion pair.[13] Rotation about the 2,3 single bond and anti allylic displacement[3] via the cisoid ion pair generates the bisabolyl cation en route to trichodiene. All operations are assumed to take place at a single enzyme active site. It is unnecessary to invoke *cis,trans*-farnesyl pyrophosphate as an intermediate, since, as previously pointed out,[13] further reaction of this substrate would proceed by way of the same ion pair from which it had been formed. Indeed, for bornyl pyrophosphate synthetase, Croteau has already demonstrated that the V_{max}/K_m for geranyl pyrophosphate is more that 20 times that for the cis isomer, neryl pyrophosphate.[11c] A coherent picture of terpenoid cyclization has therefore begun to emerge. Further investigations of the stereochemistry of farnesyl pyrophosphate isomerization–cyclization are in progress and the results will be reported in due course.[26]

Acknowledgment. 270-MHz ^1H spectra were recorded at the Southern New England High Field NMR Facility at Yale University. We should like to thank Professor Rodney Croteau for kindly sending us a preprint of ref 11a in advance of publication.

(21) B. Rickborn and R. M. Gerkin, *J. Am. Chem. Soc.*, **93**, 1693 (1971).
(22) The alternative locus of tritium, C-10, is excluded by the known derivation of H-10 from 4-H_{re} of mevalonate.[7]
(23) K. B. Sharpless and R. F. Lauer, *J. Am. Chem. Soc.*, **95**, 2697 (1973); T. Mori and K. B. Sharpless, *J. Org. Chem.*, **43**, 1689 (1978); H. J. Reich, S. Wollowitz, J. E. Trend, F. Chow, and D. F. Wendelborn, *ibid.*, **43**, 1697 (1978).
(24) (a) D. Arigoni, ref 4; (b) P. Bernasconi, Dissertation ETH Zurich No. 6024, 1977; F. Dorn, *ibid.*, No. 5554 (1975); F. Dorn and D. Arigoni, *Experientia*, **30**, 851 (1974); F. Dorn, P. Bernasconi, and D. Arigoni, *Chimia*, **29** (1), 24 (1975).
(25) See, however, H. Mackie and K. Overton, *Eur. J. Biochem.*, **77**, 101 (1977); K. H. Overton and D. J. Picken, *J. Chem. Soc., Chem. Commun.*, 105 (1976).
(26) This work was supported by NSF Grants PCM 74-07924 and PCM 78-13230.

Thermal Rearrangement of 2,2-Difluorovinylcyclopropane. A Concerted Pathway?

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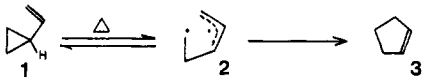
A reaction which has certainly been studied in great mechanistic detail is the rearrangement of vinylcyclopropane to cyclopentene. The bulk of the accumulated evidence is compatible with a diradical mechanism. Kinetic data (log A = 13.6, E_a = 49.7 kcal/mol)[1] are consistent with the formation of the allylically stabilized 1,3-diradical intermediate **2**, as are numerous elegant stereochemical investigations.[2] (That is not to say that "completely equilibrated" diradicals are involved, since stereoselectivity certainly is observed in a number of substituted vinylcyclopropane systems.)



As expected, radical-stabilizing substituents in the 2 position lower the activation energy for the rearrangement. For example, *trans*-2-methyl-, -phenyl-, and -methoxyvinylcyclopropanes each rearrange with cleavage of the C_1–C_2 bond and with lowered activation energies (48.6, 44.7, and 41.0 kcal/mol, respectively).[3,4] In recent years we have probed the quantitative effect of *fluorine* substituents, particularly gem-difluoro substituents, on thermal isomerizations of cyclopropane systems. The 9 kcal/mol incremental lowering of E_a for homolytic cleavage of 1,1-difluoro-2,3-dimethylcyclopropane[5] was consistent with the theoretical

(1) Flowers, F. C.; Frey, H. M. *J. Chem. Soc.* **1961**, 3547.
(2) (a) Willcott, R. M., III; Cargle, V. H. *J. Am. Chem. Soc.* **1967**, *89*, 723. (b) *Ibid.* **1969**, *91*, 4310. (c) Mazzocchi, P. H.; Tamburin, H. J. *Ibid.* **1970**, *92*, 7220. (d) Doering, W., von E.; Sachdev, K. *Ibid.* **1975**, *97*, 5512. (e) Andrews, G. D.; Baldwin, J. E. *Ibid.* **1976**, *98*, 6705.
(3) (a) Ellis, R. J.; Frey, H. M. *J. Chem. Soc.* **1964**, 5578. (b) Roth, W. R.; Konig, J. *Liebigs Ann. Chem.* **1965**, *688*, 28.
(4) Simpson, J. M.; Richey, H. G., Jr. *Tetrahedron Lett.* **1973**, 2545.

0002-7863/81/1503-2138$01.25/0