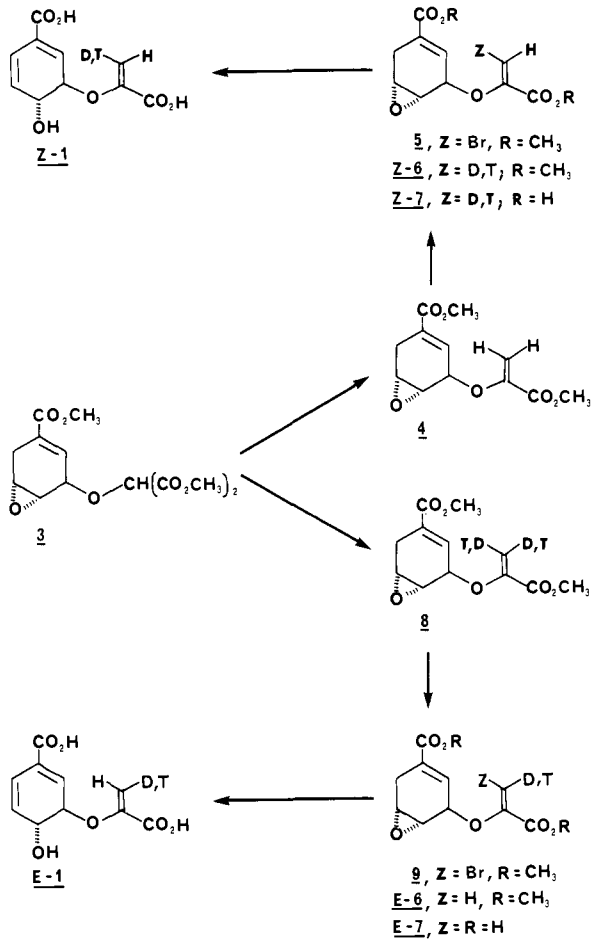
Scheme I



as the only established enzyme-catalyzed pericyclic reaction in primary metabolism.[2] In view of the rarity of [3,3]-sigmatropic rearrangements in primary metabolism, whether the enzyme-catalyzed Claisen rearrangement of **1** to **2** proceeds by a chair- or boat-like transition state is of considerable interest.

In order to establish the transition-state geometry of the enzyme-catalyzed rearrangement of **1** of **2**, it is necessary to have selective hydrogen isotope labeling of the *E* and *Z* hydrogen atoms at C-9 of **1**. Described below is our stereoselective synthesis of racemic (*E*)- and (*Z*)-[9-^{2}H,^{3}H]chorismic acid.[3]

Malonate **3** (Scheme I) was converted to **4** as described previously.[5] Bromination of **4** (Br_2, CH_2Cl_2) followed by elimination of HBr (DBN,CH_2Cl_2) gave only *Z* bromo derivative **5** (73% yield).[6] Reductive cleavage of **5** with Zn/Ag couple in THF/D_2O doped with TOH resulted in stereoselective formation of (*Z*)-**6** (93% yield; 90% (*Z*)-**6**/10% (*E*)-**6**),[7,8] The product contains ~5% unlabeled material (**4**) due to H_2O adsorbed on the catalyst. Saponification of (*Z*)-**6** (NaOH, THF/H_2O) and acidification provided the diacid ((*Z*)-**7**) with no change in distribution of the D,T label. Oxirane ring opening at C-5 with $PhSe^-$ in $CH_3OH/H_2O/NaHCO_3$[9] and subsequent selenoxide elimination with 3,5-dimethoxyaniline as scavanger as described previously for the synthesis of **1**[5] provided stereoselectively labeled (*Z*)-**1** (75% (*Z*)-**1**/25% (*E*)-**1**) and 5% unlabeled **1**.[8,10]

In order to prepare **1** with the D,T label predominantly in the *E* C-9 position, malonate **3** was subjected to Mannich reaction with $(CH_3)_2NH$ and D_2CO doped with THCO. Quaternization of the Mannich product (CH_3I, CH_2Cl_2) followed by fragmentation (NaOH, THF/H_2O) provided **8** (23% yield from **3**). Bromination of **8** and elimination of DBr(TBr) gave **9** (22% yield).[11] Reductive cleavage of **9** (Zn/Ag, THF/H_2O) produced stereoselectively labeled (*E*)-**6** (94% yield; 94% (*E*)-**6**/6% (*Z*)-**6**).[8] Treatment of (*E*)-**6** as described above gave (*E*)-**7** and, subsequently, stereoselectively labeled (*E*)-**1** (90% (*E*)-**1**/10% (*Z*)-**1**).[10]

Acknowledgment. We are grateful to J. R. Knowles and T. S. Widlanski for helpful discussion and assistance with tritium-labeling experiments and to the National Institutes of Health, Grant GM 31958, for financial support.

(2) Rétey, J.; Robinson, J. A. "Stereospecificity in Organic Chemistry and Enzymology"; Verlag Chemie: Weinheim, 1982; p 47–48.

(3) The rearrangement of (+)-**1** (unnatural enantiomer) to **2** is not catalyzed by chorismate mutase–prephenate dehydrogenase from *E. coli*, and (+)-**1** does not inhibit the enzyme-catalyzed rearrangement of (−)-**1** to **2**.[4] Consequently, labeled (±)-**1** may be used in the enzymatic investigations.

(4) Hoare, J. H.; Berchtold, G. A. *Biochem. Biophys. Res. Commun.* **1982**, *106*, 660–662.

(5) Hoare, J. H.; Policastro, P. P.; Berchtold, G. A. *J. Am. Chem. Soc.* **1983**, *105*, 6264–6267.

(6) 250-MHz ^{1}H NMR: δ 7.13 (s, 1 H); 6.87 (m, 1 H); 3.84 (s, 3 H); 3.77 (s, 3 H); 3.49 (m, 2 H); 2.88 (AB q, 2 H, J_{AB} = 20 Hz).

(7) Modified procedure of: Fryzuk, M. D.; Bosnick, B. *J. Am. Chem. Soc.* **1979**, *101*, 3043–3049 and references cited therein.

(8) The ratio was established from the (9)-**E** and (9)-**Z** proton signals in the ^{1}H NMR spectrum.

(9) When the oxirane ring of (*Z*)-**6** is cleaved with $PhSe^-$, complete scrambling of D,T label at C-9 is observed. The scrambling presumably is due to reversible Michael addition of $PhSe^-$ at C-9 of the enolpyruvate ester.

(10) We believe that the scrambling of label that occurs during (*Z*)-**7** → (*Z*)-**1** was due to the presence of unreacted 3,5-dimethoxyaniline, which underwent some reversible Michael addition during workup of the selenoxide elimination reaction. This situation was improved in the preparation of (*E*)-**1**, and the scrambling was not observed.

(11) The rate of the elimination reaction shows a significant isotope effect, and 50% of the T label is lost in the elimination reaction. The absence of an isotope discrimination in the elimination reaction suggests that the *Z* bromide is the sole product; i.e., there is no formation of *E* bromide followed by isomerization to *Z* bromide.

Stereochemistry of the Rearrangement of Chorismate to Prephenate: Chorismate Mutase Involves a Chair Transition State

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The Claisen rearrangement of chorismate (**1**) is catalyzed in bacteria and in plants by the enzyme chorismate mutase and provides the common percursor prephenate (**2**) for the essential amino acids tyrosine and phenylalanine.[1] While it is believed that in unrestricted nonenzymic systems the chair transition state for such rearrangements is favored over the boat by at least 5 kcal/mol[2] and although it has been calculated that for the chorismate–prephenate transformation a chair transition state should be favored by about 2 kcal/mol,[3,4] these energies are obviously too small to be decisive for an enzymic process the stereochemical course of which could be dominated by catalytic imperatives of much larger free energy. We have therefore de-

[†] Harvard University.
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(1) Haslam, E. "The Shikimate Pathway"; Wiley: New York, 1974.

(2) Doering, W. v. E.; Roth, W. R. *Tetrahedron* **1962**, *18*, 67. Brown, A.; Dewar, M. J. S.; Schoeller, W. W. *J. Am. Chem. Soc.* **1970**, *92*, 5516. Dewar, M. J. S.; Ford, G. P.; McKee, M. L.; Rzepa, H. S.; Wade, L. E. *Ibid.* **1977**, *99*, 5069.

(3) Andrews, P. R.; Smith, G. D.; Young, I. G. *Biochemistry* **1973**, *12*, 3492.

(4) Andrews, P. R.; Haddon, R. C. *Aust. J. Chem.* **1979**, *32*, 1921.

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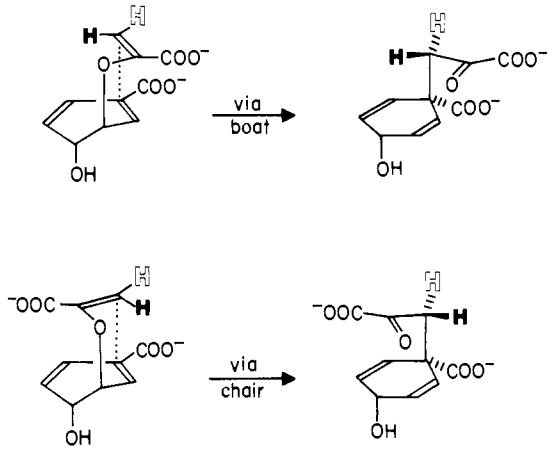
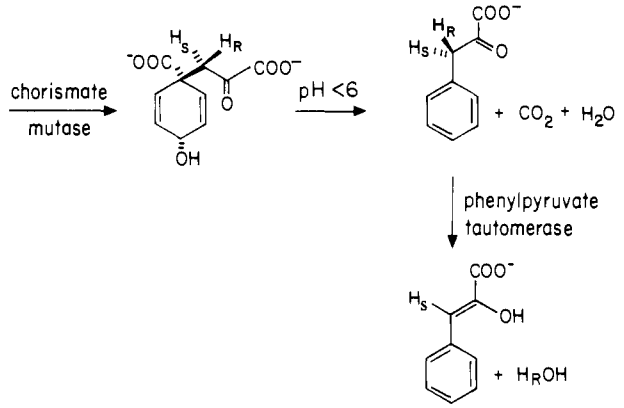
Scheme I. Transition-State Geometries for the Rearrangement of Chorismate to Prephenate[a]



[a] If the heavy hydrogen isotope is represented by the solid H, the illustrated chorismate is *Z*, the product of the boat transition state has an *R* center, and the product of the chair transition state has an *S* center.

Scheme II. Stereoanalysis of the Hydrogen Isotope Distribution in Prephenate, Using Phenylpyruvate Tautomerase



termined the nature of the transition state in the chorismate mutase reaction directly by following the rearrangement of stereoselectivity isotopically labeled chorismate. As illustrated in Scheme I, if (*Z*)-[9-^{3}H]chorismate is used as substrate, the isotopic label in prephenate creates an *R* center if the reaction proceeds through a boat transition state and an *S* center if the transition-state conformation is a chair. We report here the use of (*E*)- and (*Z*)-[9-^{3}H]chorismate, prepared either enzymically[5] or chemically,[6] as substrates for the chorismate mutase reaction, and conclude that the enzyme-catalyzed process occurs via a transition state of *chair*like geometry.

The chemically synthesized samples of chorismate were made as described earlier[6] and contained ^{1}H in one position and ^{2}H doped with ^{3}H at the other. From the ^{1}H NMR the stereochemical integrity of the samples was about 75% *Z*/25% *E* and 10% *Z*/90% *E*.[6] The enzymically synthesized samples of chorismate were derived from phosphoenolpyruvate, stereospecifically deuterated and tritiated at carbon 3.[5] The exigences of the mechanism of the synthetase that condenses phosphoenolpyruvate and shikimate-3-phosphate[5] result in chorismate samples that have ^{2}H and ^{1}H equally at both *E* and *Z* positions, while the ^{3}H tracer is about 75% *E* or *Z* (the 5-enolpyruvoylshikimate-3-phosphate synthetase reaction was run at pH 9.4). Once again, about 25% of the ^{3}H label is in the nonspecified position. As will be seen, however, both chemically derived[6] and enzymically derived[5]

(5) Grimshaw, C. E.; Sogo, S. G.; Copley, S. D.; Knowles, J. R. *J. Am. Chem. Soc.*, preceding in this issue.

(6) Hoare, J. H.; Berchtold, G. A. *J. Am. Chem. Soc.*, preceding paper in this issue.

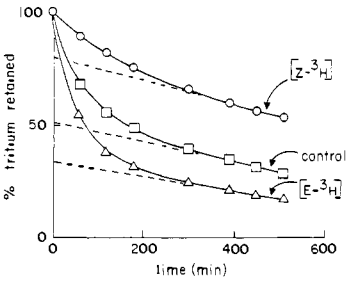


Figure 1. Rate of tritium loss from incubations of enzymically produced[5] (*Z*)-[9-^{3}H]chorismate (○), (*E*,*Z*)-[9-^{3}H]chorismate (□), and (*E*)-[9-^{3}H]chorismate (△) with chorismate mutase and phenylpyruvate tautomerase at pH <6.

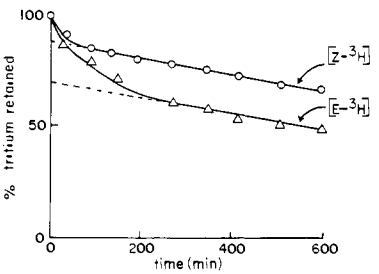


Figure 2. Rate of tritium loss from incubations of chemically synthesized[6] *Z*-[9-^{3}H]chorismate (○) and (*E*)-[9-^{3}H]chorismate (△) with chorismate mutase and phenylpyruvate tautomerase at pH 5.5.

samples of chorismate contain enough stereochemical information to allow an unambiguous solution to the problem.

A rather direct way to determine the location of ^{3}H label in the product prephenate is to use the fact that below pH 6 prephenate suffers spontaneous decarboxylative dehydration to yield phenylpyruvate[7] (Scheme II). In the presence of the enzyme phenylpyruvate tautomerase, the proton at the *pro-R* position in phenylpyruvate is preferentially labilized,[8] and any isotopic label in this position appears in the solvent. Since both chorismate mutase and phenylpyruvate tautomerase are catalytically active at pH 6, the mutase reaction and the analysis can conveniently be run together, the appearance of chorismate tritium in the solvent being monitored as a function of time.

Accordingly, [9-^{3}H]chorismate[5,6] was incubated with chorismate mutase[9] in the presence of phenylpyruvate tautomerase. When *randomly* labeled [9-^{3}H]chorismate[13] was used, a "burst" of tritium appeared in the solvent, corresponding to close to 50% of the tritium label present initially (Figure 1). When stereospecifically labeled [9-^{3}H]chorismate is used, however, the burst depends upon the tritium location in the chorismate substrate. For the enzymically produced (*Z*)-[9-^{3}H]chorismate,[5] only 20% of the tritium is lost to solvent, yet when (*E*)-[9-^{3}H]chorismate[5] is used, about 67% of the tritium is rapidly washed out (see Figure 1).[15] Analogously, chemically synthesized (*Z*)-[9-^{3}H]chorismate[6]

(7) Zamir, L. O.; Tiberio, R.; Jensen, R. A. *Tetrahedron Lett.* **1983**, *24*, 2815.

(8) Retey, J.; Bartl, K.; Engelbert, R.; Hull, W. E. *Eur. J. Biochem.* **1977**, *72*, 251.

(9) The bifunctional enzyme chorismate mutase–prephenate dehydrogenase from *E. coli* was used.[10] This enzyme has been cloned[11] and is readily isolated. In the absence of NADH, prephenate is the released product.[12]

(10) Sampathkumar, P.; Morrison, J. F. *Biochim. Biophys. Acta* **1982**, *702*, 204.

(11) We are grateful to Professor J. F. Morrison for providing the plasmid pJFM30.

(12) Sampathkumar, P.; Morrison, J. F. *Biochim. Biophys. Acta* **1982**, *702*, 212.

(13) Prepared from an incubation of unlabeled phosphoenolpyruvate and shikimate-3-phosphate, in the presence of inorganic phosphate and 5-enolpyruvoylshikimate-3-phosphate synthetase[14] in tritiated water. Under these conditions, solvent tritium is washed equally into the *E* and *Z* positions of 5-enolpyruvoylshikimate-3-phosphate.[14]

(14) Grimshaw, C. E.; Sogo, S. G.; Knowles, J. R. *J. Biol. Chem.* **1982**, *257*, 596.

loses ~13% and (*E*)-[9-^{3}H]chorismate[6] loses ~35% of the radiolabel under similar conditions (see Figure 2).[16] These data require that the chorismate mutase reaction proceeds through a chairlike transition state[18] in which the *Z* proton of chorismate becomes the *pro-S* proton of prephenate.

Acknowledgment. We are grateful to Steven Benner, who first suggested phenylpyruvate tautomerase as a simpler route than phenylalanine ammonia lyase[17] for the analysis of the product tritium distribution, and to the National Institutes of Health and Merck Sharp & Dohme for support.

(15) It should be noted that the absolute value of the extrapolated burst is rather imprecise, since a compromise between a pH low enough for rapid decarboxylative dehydration of prephenate yet high enough for reasonable mutase and tautomerase activity is necessary. The *relative* rate of tritium washout from the *Z* and *E* isomers is, however, unambiguous.

(16) The synthetic chorismate[6] is racemic, so only half of the chorismate sample is taken to prephenate by chorismate mutase. The percentage bursts are therefore 26% and 70% of the natural enantiomer.

(17) Ife, R. J.; Ball, L. F.; Lowe, P.; Haslam, E. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1776.

(18) Our results provide proof for the suggestion made earlier on the basis of studies of the inhibition of chorismate mutase by various dicarboxylates: Andrews, P. R.; Cain, E. N.; Rizzardo, E., Smith, G. D. *Biochemistry* **1977**, *16*, 4848.

Cyclomevalonic Acid

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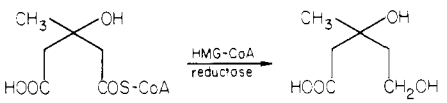
Reduction of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) (I) to mevalonic acid (II) by HMG-CoA reductase (EC 1.1.1.34)

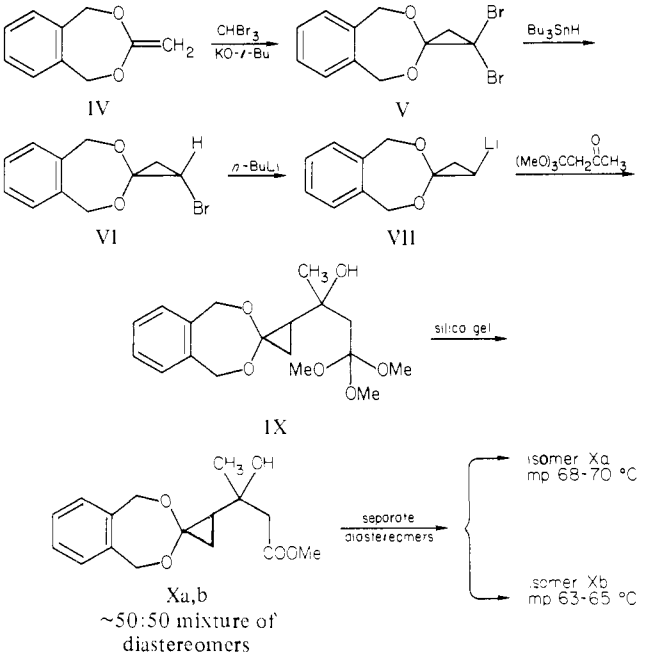


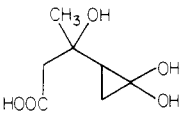
I II

is the rate-limiting step in the biosynthesis of cholesterol.[1,2] As a consequence, the discovery of substances capable of specifically inhibiting this enzymic transformation[3] is important.

Reduction by HMG-CoA reductase occurs at an active-site thiol whose role is analogous to the active-site thiol in aldehyde dehydrogenase. The latter has been shown to be inhibited by cyclopropanone hydrate, which is, in size, comparable to acetaldehyde.[4] Accordingly, incorporation of a cyclopropanone hydrate into mevalonic acid, to learn whether such a strategm might lead to an inhibitor of HMG-CoA reductase, became attractive. This communication describes the synthesis of cyclomevalonic acid (III), a novel derivative of mevalonic acid (II).

Scheme I





III

It was recognized that cyclomevalonic acid (III) would be a sensitive substance and that the unmasking of the cyclopropanone hydrate[5] should be carried out under mild conditions and be the final step in the synthesis.

The synthetic scheme is outlined in Scheme I: The ketene acetal IV[6] was treated with bromoform and potassium *tert*-butoxide[7] in pentane yielding (75%) the dibromocyclopropane V, mp 136–137 °C.[8] Treatment of the dibromide V with tri-*n*-butyltin hydride[9,10] in benzene yielded (60%) the monobromide VI,[8] mp 75–76 °C. The monobromide VI in ether was treated with *n*-BuLi for 2.5 h at −78 °C generating the lithiated cyclopropane VII. Reaction of VII with 1,1,1-trimethoxybutan-3-one (VIII) followed by quenching at −78 °C with water yielded the adduct IX. Purification of IX by column chromatography on silica gel resulted

(1) Reviews: Rodwell, V. W.; Nordstrom, J. L.; Mitschelen, J. J. *Adv. Lipid Res.* **1976**, *14*, 1. Rodwell, V. W.; McNamara, D. J.; Shapiro, D. J. *Adv. Enzymol.* **1973**, *38*, 373.

(2) Lehninger, A. L. "Biochemistry", 2nd ed.; Worth: New York, 1975; p 681.

(3) Endo, A.; Kuroda, M.; Tanzawa, K. *FEBS Lett.* **1976**, *72*, 323. Endo, A.; Kuroda, M.; Tsujita, Y. *J. Antibiot.* **1976**, *29*, 1346. Brown, A. G.; Smale, T. C.; King, T. J.; Hasenkampe, R.; Thompson, R. H. *J. Chem. Soc., Perkin Trans. 1* 1165 (1976). M. S. Brown, J. R. Faust, J. L. Goldstein, L. Kaneko, and A. Endo, A. *J. Biol. Chem.* **1978**, *253*, 1121. Brown, M. S.; Goldstein, J. L. *Ibid.* **1974**, *249*, 7306. Kandutsch, A. A.; Chen, H. W. *Ibid.* **1974**, *249*, 6075. Nelson, J. A.; Czarny, M. R.; Spencer, T. A.; Limanek, J. S.; K. R. MacCrae, K. R.; Chang, T. Y. *J. Am. Chem. Soc.* **1978**, *100*, 4900 and references cited therein. Schroepfer, G. J., Jr.; Parish, E. J.; Gilliland, G. L.; Newcomer, M. E.; Somerville, L. L.; Quiacho, F. A.; Kandutsch, A. A. *Ibid.* **1978**, **84**, 823. Kuroda, M.; Ando, A. *Biochim. Biophys. Acta* **1977**, *486*, 70. George, R. J.; Menon, A. S.; Ramasarma, T. *Biochem. Biophys. Res. Commun.* **1978**, *84*, 544 and references cited therein.

(4) Wiseman, J. S.; Abeles, R. H. *Biochemistry* **1979**, *18*, 427. Wiseman, J. S.; Tayrien, G.; Abeles, R. H. *Ibid.* **1980**, 19, 4222. Lindberg, P.; Bergman, R.; Wickberg, B. *J. Chem. Soc., Perkin Trans. 1* **1977**, 684. Tottmar, O.; Lindberg, P. *Acta Pharmacol. Toxicol.* **1977**, *40*, 476.

(5) Cyclopropanone reviews: Wasserman, H. H.; Clark, G. M.; Turley, P. C. *Fortschr. Chem. Forsch.* **1974**, *47*, 73. Turro, N. J. *Acc. Chem. Res.* **1969**, *2*, 25. See also: Turro, N. J.; Hammond, W. B. *Tetrhedron* **1968**, *24*, 6017; 6029. Schaafsma, S. E.; Steinberg, H.; de Boer, Th. J. *Recl. Trav. Chim. Pays-Bas* **1966**, *85*, 1171. Tilborg, W. J. M.; Schaafsma, S. E.; Steinberg, H.; de Boer, Th. J. *Ibid.* **1967**, *86*, 417.

(6) Grewe, R.; Struve, A. *Chem. Ber.* **1963**, *96*, 2819.

(7) Doering, W. von E.; Hoffmann, A. K. *J. Am. Chem. Soc.* **1954**, *76*, 6162.

(8) All new substances showed satisfactory NMR, IR, and mass spectra, including exact mass determination.

(9) Kuivila, H. G. *J. Org. Chem.* **1961**, *25*, 284. Kuivila, H. G.; Walsh, E. J., Jr. *J. Am. Chem. Soc.* **1966**, **88**, 571. Kuivila, H. G.; Menapace, L. W.; Warner, C. R. *Ibid.* **1962**, *84*, 3584.

(10) Seyferth, D.; Yamazaki, H.; Alleston, D. L. *J. Org. Chem.* **1963**, *28*, 703.

0002-7863/84/1506-2703$01.50/0