tralized with a 20% sodium hydroxide solution, extracted with ether, and dried over anhydrous magnesium sulfate. Distillation of this solution through a 6-in. Vigreux column gave 34 g (32%) of **8a**: bp 80–82° (0.6 mm); infrared absorption (smear), 5.63 μ; nmr spectrum (neat), multiplet at 0.90 (methyl groups), multiplet at 1.36 (four methylene groups), singlet at 2.20 (dimethylamino group), multiplet at 2.32 (methylidyne proton), multiplet at 2.75 (methylene group in ring), and multiplet at 2.41 ppm (methylene group adjacent to nitrogen).

Anal. Calcd for $C_{13}H_{25}NO$: C, 74.0; H, 11.8; N, 6.6. Found: C, 73.9; H, 12.2; N, 6.9.

2-Butyl-2-ethyl-3-(piperidinomethyl)cyclobutanone (8b).—Under the same conditions used for **8a**, butylethylketene and N-allylpiperidine gave **8b** in 22% yield: bp 98–101° (0.15 mm); infrared absorption (smear), 5.62 μ.

Anal. Calcd for $C_{16}H_{29}NO$: C, 76.4; H, 11.6; N, 5.6. Found: C, 75.8; H, 11.6; N, 5.8.

2-Butyl-2-ethyl-3-(morpholinomethyl)cyclobutanone (8c).—Under the same conditions used for **8a**, butylethylketene and N-allylmorpholine gave 28% of **8c**: bp 108–110° (0.1 mm); infrared absorption (smear), 5.64 μ.

Anal. Calcd for $C_{15}H_{27}NO_2$: C, 71.1; H, 10.7; N, 5.5. Found: C, 70.8; H, 10.8; N, 5.7.

2,2-Dimethyl-3-(N-acetylpropylamino)cyclobutanone (9).—To a stirred solution of 55.9 g (0.44 mole) of N-propyl-N-vinylacetamide in 200 ml of benzene was added 35 g (0.5 mole) of dimethylketene under nitrogen. The reaction temperature slowly rose to 35°. After being stirred for 5 hr, the solution was distilled through a 12-in. Vigreux column to obtain some unchanged N-propyl-N-vinylacetamide, tetramethyl-1,3-cyclobutanedione, and 42.0 g (48%) of **9**: bp 126–128° (1.5 mm); $n^{20}D$ 1.4758; infrared absorptions (smear), 5.65 and 6.13 μ; nmr spectrum (neat), triplet at 0.93 (methyl of propyl group), two peaks at 0.94 and 1.26 (*gem*-dimethyl group), a singlet at 2.05 (methyl of acetyl group), multiple peaks at 1.25 and 1.93 (middle methyl of propyl group), multiple peaks from 3.11 to 3.52 (methylene of ring and methylene in propyl group adjacent to nitrogen), and triplet at 4.03 ppm (methylidyne proton).

Anal. Calcd for $C_{11}H_{19}NO_2$: C, 67.0; H, 9.7; N, 7.1. Found: C, 66.8; H, 10.0; N, 7.1.

2,2-Dimethyl-3-(N-acetylpropylamino)cyclobutanol (11).—A solution of 30 g of the cyclobutanone **9** in 70 ml of ethyl alcohol was hydrogenated in a rocking autoclave at 100° and 3000 psi over 5 g of a 5% ruthenium-on-carbon (powdered) catalyst. This reaction solution was filtered to remove the catalyst, and the filtrate was distilled through a 10-in. packed column to give 25.2 g (81%) of **11**: bp 143° (0.8 mm); $n^{20}D$ 1.4845; infrared absorptions (smear), 2.95 and 6.15 μ.

Anal. Calcd for $C_{11}H_{21}NO_2$: C, 66.4; H, 10.5; N, 7.0. Found: C, 66.2; H, 10.7; N, 6.9.

2,2-Dimethyl-3-(N-benzoylpropylamino)cyclobutanol (12).—To a solution of 12.3 g (0.05 mole) of 2,2-dimethyl-3-(N-benzoylpropylamino)cyclobutanone in 30 ml of ethyl alcohol was added slowly with stirring a solution of 0.76 g (0.02 mole) of sodium borohydride in 5 ml of water. The reaction solution was stirred for 1 hr at room temperature and then evaporated on a steam bath. The residue was taken up in ether, washed with water, and dried over anhydrous sodium sulfate. Distillation of this solution gave 9.8 g (80%) of **12** as a clear, very viscous distillate: bp 192–196° (1 mm); infrared absorptions (smear), 3.0 and 6.2 μ.

Anal. Calcd for $C_{16}H_{23}NO_2$: C, 73.6; H, 8.8; N, 5.4. Found: C, 73.4; H, 8.8; N, 5.3.

The Reaction of Butylethylketene with N-Propyl-N-vinylacetamide.—A solution of 51 g (0.4 mole) of N-propyl-N-vinylacetamide and 50 g (0.4 mole) of butylethylketene in 100 ml of hexane was refluxed for 8 hr. The infrared spectrum of this solution had a strong band at 5.63 μ (cyclobutanone). The solution was distilled through a 12-in. packed column to recover about 20 g of unchanged butylethylketene dimer and 45 g of a mixture of unchanged N-propyl-N-vinylacetamide and butylethylketene dimer, bp 64–107° (0.15 mm). The product (26 g) was taken at 107–133° (0.15 mm). This material was redistilled through a spinning-band column to give 5.1 g of N-(4-ethyl-3-oxo-1-octen-1-yl)-N-propylacetamide (**14**): bp 126° (0.2 mm); $n^{20}D$ 1.5022; infrared absorptions (smear), 5.9, 6.18, and 6.3 μ; nmr spectrum (CCl_4), a pair of doublets at 5.75 and 8.04 ppm

$$\begin{matrix} & & O & & \\ & & \| & & \\ R & C & C & H{=}CHNR_2 & \end{matrix}$$

(the protons of the RCCH=CHNR$_2$ grouping).

Anal. Calcd for $C_{15}H_{27}NO_2$: C, 71.1; H, 10.7; N, 5.5. Found: C, 71.0; H, 10.7; N, 5.5.

N-Methyl-N-(2,2-dimethyl-3-oxocyclobutyl)benzenesulfonamide (15a).—To a stirred solution of 197 g (1.0 mole) of N-methyl-N-vinylbenzenesulfonamide in 500 ml of acetonitrile under nitrogen was added 70 g (1.0 mole) of dimethylketene. The reaction temperature was held at 25–35° by a cooling bath. After being stirred for several hours, the reaction solution was distilled through a 12-in. Vigreux column to recover some tetramethyl-1,3-cyclobutanedione and 127 g of unchanged N-methyl-N-vinylbenzenesulfonamide, bp 113–114° (0.8 mm). The distillation was continued in a molecular still to give 65 g (24%) of **15a**, bp 93–102° (2–3 μ), $n^{20}D$ 1.5429. This distillate slowly crystallized on cooling. A sample recrystallized from ethyl alcohol had mp 58–60°; infrared absorption (KBr), 5.62 μ; nmr spectrum (CCl_4), doublet at 1.18 (*gem*-dimethyl group), singlet at 2.70 (methyl group on nitrogen), multiple peaks between 2.81 and 3.92 (methylene and methylidyne groups of cyclobutane ring), and multiplet at 7.52 ppm (aromatic protons).

Anal. Calcd for $C_{13}H_{17}NO_3S$: C, 58.4; H, 6.4; N, 5.2; S, 12.0. Found: C, 58.4; H, 6.4; N, 5.2; S, 12.2.

Synthesis and Hydrolysis of α- and ϵ-Peptides of Lysine[1]

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In order to compare ϵ-peptide bonds with α-peptide bonds, dipeptides of L-lysine were synthesized by coupling glycine, L-alanine, L-phenylalanine, L-leucine, or L-aspartic acid to the α- or ϵ-amino group of lysine by the mixed carboxylic–carbonic acid anhydride method. The first-order rate constants of hydrolysis of these peptides were studied in 3 *N* and 6 *N* HCl and NaOH at 55, 75, and 97°. Except for the aspartyllysines, there were only slight differences in the rates of acid-catalyzed hydrolysis of the corresponding α- and ϵ-peptides under various experimental conditions. In base-catalyzed hydrolysis, ϵ-peptides were hydrolyzed 4–9.5 times faster than α-peptides showing that the position of the peptide bond as well as the side chain of the amino acid coupled to lysine determine the rate of hydrolysis.

In proteins the amino group on the side chain of lysine residues offers the possibility of an alternate structure involving the ϵ-amino group in addition to or instead of the α-amino group in chemical linkage to the adjacent amino acid. In the former case a branched chain protein is formed as observed in bovine growth hormone[3] and collagen.[4] Polypeptidyl proteins in which branched chains are built onto a protein mole-

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TABLE I

SYNTHESIS AND PROPERTIES OF DERIVATIVES OF PEPTIDES OF LYSINE AND OF FREE PEPTIDES

	R_f		Mp, °C		% nitrogen		
	Lit.	Found	Lit.	Found	Calcd	Found	$[\alpha]^{24.5-26.5}D$, deg
ε-Cbz[a]-Lys-Bz[b]·HCl		0.94	139[c]	138			
α-Cbz-Gly, ε-Cbz-Lys-Bz[d]		0.93	87–89[d]	87			
α-Cbz-Ala, ε-Cbz-Lys-Bz[d]		0.93		112–113			
α-Cbz-Phe, ε-Cbz-Lys-Bz[e]		0.93		155–158			
α-Cbz-Leu, ε-Cbz-Lys-Bz[f]		0.93		82[g]			
α-(α-Cbz-Asp-β-Bz), ε-Cbz-Lys-Bz[h]		0.93		62[g]			
α-Gly-Lys·HOAc[i]	0.18[i]	0.14			15.95	15.64	+4.33
α-Ala-Lys·HOAc[i]		0.18			15.15	15.22	+9.63
α-Phe-Lys·HOAc[i]		0.39			11.88	12.14	+5.43
α-Leu-Lys·HOAc[i]		0.38			13.14	13.12	+25.03
α-(α-Asp)-Lys[i]		0.09			16.07	16.35	+30.60
ε-Gly-Lys·HOAc[j]	0.20[j]	0.18			15.95	16.05	+15.70
ε-Ala-Lys·HOAc[j]		0.21			15.15	15.13	+7.60
ε-Phe-Lys·HOAc[j]	0.44[j]	0.42			11.88	12.08	+27.97
ε-Leu-Lys·HOAc[k]		0.40			13.14	12.94	+47.97
ε-(α-Asp)-Lys[j]		0.10			16.07	16.14	+11.60

[a] L-Amino acids were used. Abbreviations used are Cbz, carbobenzoxy; Bz, benzyl; amino acid residues as given by Brand.[15] [b] According to the method of Boissonnas, *et al.*,[16] the derivative being precipitated with anhydrous ether. [c] See ref 18. [d] According to the procedure of Levin, *et al.*,[17] with washing of the organic phase with 3% $NaHCO_3$ and water. [e] As in *d*, obtained from the precipitate and from the solution. [f] As in *d*, with additional washings with 1 *N* HCl. [g] Softens. [h] As in *d*, Cbz-Asp-β-Bz ester (Yeda, New England Nuclear Corp., Boston, Mass.) was used as intermediate. [i] By the method of Erlanger and Brand,[18] hydrogenolysis in presence of acetic acid and Pd black for 4 hr. [j] By the method of Theodoropoulos,[19] hydrogenolysis within 2 hr. [k] As in *j*, hydrogenolysis within 4 hr, recrystallized from glacial acetic acid–anhydrous ether.

cule by reaction of an N-carboxyamino acid anhydride with the α- and ε-amino groups of the protein[5] have been employed for investigations of physical,[6-8] immunological,[9] and enzymatic[10] properties of proteins.

The ε-amino groups of lysine and hydroxylysine in collagen have been implicated in the calcification process.[11] The ε-amino group has also been found in amide linkage with nonamino acids as in the binding of biotin[12] and lipoic acid.[13] It can also be in a peptide bond with a side-chain carboxylic group of a dicarboxylic amino acid to form a cyclic peptide as in bacitracin A.[14] Thus it is possible to have branch points or cross-links other than disulfide bridges in protein molecules.

To compare the nature of ε-peptide bonds with α-peptide bonds, dipeptides were synthesized by coupling glycine, L-alanine, L-phenylalanine, L-leucine, or L-aspartic acid to the α- or ε-amino group of L-lysine and the rates of hydrolysis of the peptides by acid and alkali were studied under various experimental conditions. In the course of this study, some peptides and peptide derivatives not previously reported were prepared.

Experimental Section

Preparation of α- and ε-Peptides of Lysine.—These were synthesized by the mixed carboxylic–carbonic acid anhydride procedure using the N-carbobenzoxy derivative of the desired amino acid and lysine benzyl ester for α-peptides or lysine copper complex for ε-peptides. The derivatives were converted to the free peptides by catalytic hydrogenation. The materials prepared for this study are given in Table I.

Paper Chromatographic Analysis.—Compounds to be tested for purity were dissolved in water or ethyl acetate and applied on Whatman No. 1 paper (56.5 × 18 cm). They were analyzed by descending chromatography using the solvent mixture 1-butanol–acetic acid–pyridine–water (30:6:20:24, v/v).[17] The presence of NH groups was detected according to the method of Mazur, *et al.*[20] The carbobenzoxy derivatives of peptide esters and carbobenzoxylysine benzyl ester have the same mobility; to distinguish them, these chromatograms were sprayed with an alcoholic solution of ninhydrin. Only ε-carbobenzoxylysine benzyl ester developed color while the carbobenzoxy derivatives of peptide benzyl esters did not since the NH_2 groups were acylated.

Percentage of Nitrogen in Peptides of Lysine.—Percentage of nitrogen was determined by the microKjeldahl method of Lang.[21]

Optical Rotations of Peptides of Lysine.—Optical rotations of 0.5% solutions of the peptides in demineralized water were taken on a Rudolph photoelectric spectropolarimeter (Model 200) with an oscillating polarizer (Model 340) using the D line of sodium at 589 mμ.

Colorimetric Assay for Lysine.—Free lysine was assayed according to the method of Work,[22] using water as the blank; a straight line was obtained up to 1.5 μmoles of lysine. The lysine liberated during acidic and alkaline hydrolysis of lysine compounds was assayed according to this method; appropriate blanks were included in each assay. The absorbancies were read at 340 mμ on a Hitachi Perkin-Elmer 139 UV VIS spectrophotometer or on a Zeiss PMQ II spectrophotometer in a 1-cm cell.

Acidic and Basic Hydrolysis of Peptides of Lysine.—Sufficient α- or ε-peptide to make 3 μmoles/ml was weighed and dissolved in 10 ml of 3 *N* or 6 *N* HCl or NaOH. Aliquots (1 ml) were pipetted into separate test tubes, cooled in ice, sealed, and incubated at 55, 75, or 97° in an oil bath (Fisher Hi temp bath) except those for zero-time readings. Tubes were withdrawn from

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TABLE II

RATE CONSTANTS FOR THE HYDROLYSIS OF PEPTIDES OF LYSINE

		$k_1 \times 10^{-4}$ sec^{-1}									
		Gly-Lys		Ala-Lys		Phe-Lys		Leu-Lys		(α-Asp)-Lys	
Solvent	Temp, °C	α	ε	α	ε	α	ε	α	ε	α	ε
3 *N* HCl	97	0.95	0.92							1.18	0.95
6 *N* HCl	75	0.77	0.65	0.31	0.32					0.60	0.28
	97	1.92	1.58	0.86	1.02	0.28	0.31	0.33	0.46	1.68	1.08
3 *N* NaOH	55	0.87	5.77								
	75			0.53	2.81	0.15	1.10			0.36	2.56
	97							0.34	2.80		
6 *N* NaOH	55			0.38	2.54	0.15	1.09			0.34	3.25
	75			1.47	5.93			0.44	2.77		

the bath at definite intervals of time and plunged into ice to stop the hydrolysis. The tubes were cracked open and 1 ml of 3 *N* or 6 *N* HCl or NaOH was added to neutralize the solution. Duplicate samples were analyzed for free lysine. Readings for complete hydrolysis were obtained from three samples containing 3 μmoles of the peptide/ml of 6 *N* HCl heated at 120–125° for 48 hr and then neutralized by 6 *N* NaOH. Averages of the triplicate readings were taken.

Results

Paper Chromatographic Analysis.—Only single spots were detected upon paper chromatographic analysis of the free amino acids, amino acid derivatives, peptide derivatives, and free peptides employed in this study.

Acid- and Base-Catalyzed Hydrolysis.—The absorbancy at 340 mμ is proportional to the concentration of lysine in solution; therefore, in an excess of acid or alkali, the integrated first-order rate equation for the release of lysine by hydrolysis of a dipeptide is $\ln [(A_\infty - A_0)/(A_\infty - A_t)] = k_1 t$, where A_0, A_t, and A_∞ are absorbancies at time 0, t, and complete hydrolysis, respectively, and k_1 is the hydrolysis constant.[23] In order to get a measurable rate of hydrolysis for each peptide, temperatures of 55, 75, and 97° and acid and alklai concentrations of 3 and 6 *N* were employed. Hydrolysis of phenylalanine and leucine peptides proceeded to a measurable degree in 6 *N* HCl at 97°, while the rates of hydrolysis of other peptides were high enough at lower concentrations of acid and at lower temperatures. The rates of hydrolysis of ε-peptides in alkali were always much higher than α-peptides; therefore, suitable temperatures and concentrations of alkali had to be selected to follow the rates of hydrolysis.

First-order kinetics were observed in acid- and base-catalyzed hydrolysis of the α- and ε-peptides of lysine. The calculated rate constants under appropriate hydrolytic conditions are given in Table II. There are only very slight differences in the rates of acid-catalyzed hydrolysis of the corresponding α- and ε-peptides of lysine except the aspartyllysines. In base-catalyzed hydrolysis, the ε-peptides are hydrolyzed 4–9.5 times faster than the corresponding α-peptides.

Discussion

α- and ε-peptides of lysine were synthesized by known procedures with some modifications. In most of the cases the yields of the carbobenzoxy derivatives of peptides and peptide esters were over 60% and the yields of peptides by catalytic hydrogenolysis were 70–96%. Evidence for complete removal of unreacted intermediates was obtained by paper chromatography. The individual methods for preparation of amino acid derivatives or for isolation of the peptides were found to be necessary in order to prepare chromatographically pure peptides.

The protonation of α- and ε-peptides takes place to the same extent in a strong acid medium. The presence of an electron attracting carboxylic group, one in α-glycyllysine and two in α-(α-aspartyl)lysine, near the reaction site facilitates the removal of the oxonium ion. The difference in k_1 values of α- and ε-(α-aspartyl)-lysine is higher than the difference of k_1 values of α- and ε-glycyllysine. This suggests the participation of two carboxylic groups in the hydrolysis of α-(α-aspartyl)lysine. The unusual stability of ε-(α-aspartyl)lysine toward acid hydrolysis is due to the formation of the cyclic intermediate ε-(α-aminosuccinyl)lysine which is more resistant to strong acid than α-(α-aminosuccinyl)lysine.[24]

ε-(α-Aspartyl)lysine[25] and ε-(α-glycylglutamyl)lysine[4] were isolated from bacitracin A and collagen, respectively, after partial hydrolysis with HCl. This is in accordance with the finding that ε-peptide bonds involving dicarboxylic amino acids are more stable in acid-catalyzed hydrolysis than the comparable α-peptide bonds.

The electron-repelling inductive effect of the hydrocarbon chain and the electron-attracting inductive effect of the carboxylic group of α-alanyllysine and α-leucyllysine are operating in opposite directions and their inductive effects are not likely to influence the rates of hydrolysis. The slight increase in k_1 values for the hydrolysis of ε-alanyllysine and ε-leucyllysine over their corresponding α-peptides may be a result of steric effects. That the k_1 values for the hydrolysis of leucyllysine and phenylalanyllysine are much lower than those for the corresponding alanyllysine or glycyllysine may be accounted for by greater steric effects of the side-chain groups. It is to be noted that in ε-alanyllysine and ε-leucyllysine, the hydrocarbon chains by their electron-releasing inductive effects may stabilize the protonated substrate, thus decreasing the rates of hydrolysis. The rates of base-catalyzed hydrolysis of all ε-peptides are 4–9.5 times higher than their corresponding α-peptides. These differences in rates of hydrolysis are perhaps caused by steric hindrance of the substituents on the α-carbon atom of the N-terminal amino acid in α-peptides.

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It may be concluded from the data presented that (1) the rate of hydrolysis is affected greatly by the amino acid coupled with lysine; (2) ϵ-peptide bonds are more labile than α-peptide bonds to alkaline hydrolysis; (3) there is only a slight difference between the rates of acid-catalyzed hydrolysis of corresponding α- and ϵ-peptide bonds except those involving dicarboxylic amino acids.

Reactions of the Limonene 1,2-Oxides. I. The Stereospecific Reactions of the (+)-*cis*- and (+)-*trans*-Limonene 1,2-Oxides

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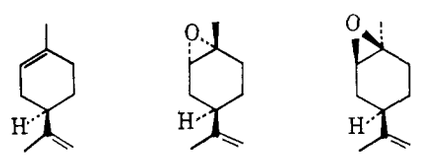
Pure *cis*- and *trans*-limonene 1,2-oxides have been prepared, and certain of their reactions with nucleophilic and with electrophilic reagents have been studied in detail. It has been shown that the Fürst–Plattner rule predicts the predominant products in all cases studied. Studies of the pyrolysis of hydroxyacetates derived from the limonene 1,2-epoxides of known configurations have permitted confirmation of the correct configurations in the carvomenthol series.

The limonene 1,2-oxides, which occur naturally in the essential oils of *Cymbopogon densiflorus*,[2] afford one of the most ideal cases yet investigated for study of the various competing effects which determine the stereochemistry of the opening of the oxirane linkage in substituted monocyclic cyclohexene epoxides.

The stereospecific reactions of cyclohexene epoxides in fused-ring systems, such as steroid epoxides and certain sugar epoxides, have been shown to yield *trans* diaxial products.[3] This rule has been stated for the steroids by Fürst and Plattner[5] and extended to the sugar epoxides by Mills.[6] The case of substituted monocyclic cyclohexene epoxides has not been as thoroughly studied and to assume that *trans* diaxial products will always be the rule would be unwise, since conformational effects in such systems are not always as clear-cut as in the case of fused-ring systems. In resolving this question, three major effects must be considered: (a) conformational effects on the cyclohexene epoxide,[7] (b) the primary steric effect[4] (steric hindrance), and (c) conformational preference of the resultant products.

The effect of bulky[8,9] alkyl groups in anchoring the ring conformation of substituted monocyclic cyclohexene epoxides has been established by their adherence to the Fürst–Plattner rule (*i.e.*, the 4-*t*-butylcyclohexene epoxides[10]), while interconversion of the two possible epoxide conformations has been demonstrated in the anhydroinositols.[11] Angyal has contended that the apparent exceptions to the Fürst–Plattner rule in monocyclic compounds are not due to *equatorial opening*, but rather to the reaction of the epoxide in its alternate conformation with subsequent inversion of the *diaxial* product to the more stable diequatorial conformation. Angyal warned, however, that predictions about the direction of ring opening might not always be reliable, because it would be difficult in some cases to presuppose the conformation in which the epoxide would react.[11]

The epoxidation of alkyl-substituted monocyclic cyclohexenes has been generally assumed to give epoxides which are predominantly *trans* to the alkyl substituents.[12,13] However, from an examination of models of 4-alkylcyclohexenes, it is not apparent that one side is more hindered than the other and thus,



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(7) The epoxide ring tends to "flatten out" the cyclohexane ring in the same way as an olefinic linkage, causing the cyclohexane ring to assume a half-chair conformation [B. Ottar, *Acta Chem. Scand.*, **1**, 283 (1947)]. It has been assumed that the stable chair conformation of the cyclohexane ring is partially reestablished in the transition state.

(8) The observation that the small hydroxyl group can anchor the ring conformation of epoxycyclohexanols in their reactions with lithium aluminum hydride is presumably the result of the formation of a complex by the hydroxyl with the aluminum hydride. This can, in some cases, lead to anomalous results.[9]

(9) H. B. Henbest and R. A. L. Wilson, *Chem. Ind.* (London), 659 (1956); *J. Chem. Soc.*, 1958 (1957).

(10) J. Sicher, F. Šipoš, and M. Tichý, *Collection Czech. Chem. Commun.*, **26**, 847 (1961).

(11) S. J. Angyal, *Chem. Ind.* (London), 1230 (1954); *Quart. Rev.* (London), **11**, 212 (1957); see also R. C. Cookson, *Chem. Ind.* (London), 223, 1512 (1954).

(12) E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, pp 293–294, and references therein.

(13) For discussion and leading references on the directing influence of substituents in epoxidation reactions, see H. B. Henbest, *Proc. Chem. Soc.*, 159 (1963).