TABLE III

PREPARATION OF DL-3-CARBOXY-4-AMINO-N-ALKYLBUTYRAMIDES

Substance,[d] 3-carboxy-4-Amino-N-alkyl-butyramide	M.p., °C.	R_f	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
n-Butyl[a]	220	0.92	$C_9H_{18}N_2O_3$	53.5	53.2	8.9	9.1	13.9	14.1
Isobutyl[a]	232	0.90	$C_9H_{18}N_2O_3$	53.5	52.9	8.9	9.0	13.9	13.9
n-Hexyl[a]	234	0.94	$C_{11}H_{22}N_2O_3$	57.4	56.9	9.6	9.3	12.2	12.1
Cyclohexyl[a]	231	0.94	$C_{11}H_{20}N_2O_3$	57.9	58.6	8.8	9.0	12.3	12.4
Carbethoxymethyl[b,c]	202	0.94	$C_9H_{16}N_2O_5$	46.6	46.2	6.9	7.0	12.1	12.3

[a] Obtained in approximately quantitative yield. [b] Recrystallized from water–acetone. [c] Obtained in 80% yield. [d] Substances were recrystallized from water if not indicated otherwise.

Itaconic anhydride was prepared in 92% yield by heating itaconic acid with acetyl chloride,[15] m.p. 65°.

Preparation of 2-Methylene-N-alkylsuccinamic Acids.—To an ice-cooled solution of 0.1 mole of itaconic anhydride in 60 ml. of dry chloroform, 0.1 mole of amine was added dropwise with mechanical stirring during 15 min. The reaction mixture was stirred for 2 hr. at room temperature, and the product was filtered and washed with chloroform. Another crop (5–20% yield) was obtained on evaporation of the filtrate.

With amino acid esters, the reaction mixture was stirred for another hour at 40°. The chloroform was evaporated *in vacuo;* the residue was dissolved in a small volume of ethyl acetate and left to crystallize in a refrigerator.

The products gave a positive reaction for double bonds with aqueous permanganate solution and a negative reaction with ninhydrin.

2-Methylenesuccinamic Acid.—Excess dry gaseous ammonia was passed into an ice-cooled solution of itaconic anhydride in chloroform. The ammonium salt which precipitated was filtered, dissolved in water, and heated for a few minutes to expell excess ammonia. The solution was then passed through a column packed with cation exchange resin (nuclear sulfonic acid type resin, Amberlite IR-120) and evaporated *in vacuo*. The product which crystallized was obtained in 40% yield. It was recrystallized from ethanol, m.p. 152°.

Anal. Calcd. for $C_5H_7NO_3$: C, 46.5; H, 5.4; N, 10.8. Found: C, 46.9; H, 5.5; N, 10.8.

Preparation of DL-3-Carboxy-4-benzylamino-N-alkylbutyramides.—2-Methylene-N-alkylsuccinamic acid (0.1 mole) was suspended in 70 ml. of dry dioxane and heated under reflux and mechanical stirring until it dissolved. Benzylamine (0.1 mole) was then added and the reaction mixture heated in an oil bath at 120° for 2–3 hr. The reaction product generally started to precipitate within 15–30 min. The reaction mixture was cooled and the product was filtered and washed with acetone. An additional crop was obtained on evaporation of the filtrate and recrystallization of the residue from alcohol, acetone, or water.

The pure products gave negative permanganate and ninhydrin reactions.

Preparation of DL-3-Carboxy-4-amino-N-alkylbutyramides.—DL-3-Carboxy-4-benzylamino-N-alkylbutyramide (0.02 mole) was suspended in 120 ml. of absolute ethanol and 0.4 g. of catalyst (palladium chloride on charcoal, 30%, was added). The hydrogenolysis was carried out in a Parr low pressure hydrogenation apparatus for about 16 hr. at 50–60°. The product generally precipitated on the catalyst from which it was extracted with boiling water. The free amino acids generally crystallized out on cooling.

DL-3-Carboxy-4-dibenzylaminobutyric Acid.—Itaconic acid (13 g., 0.1 mole) was dissolved in 50 ml. of dry dioxane, dibenzylamine (19.7 g., 0.1 mole) was added, and the reaction mixture was heated for 4 hr. at 120°. The dioxane was evaporated *in vacuo;* the residue was dissolved in a small volume of ethanol and left to crystallize in a refrigerator; yield, 19 g. (58%); m.p. 148°, on recrystallization from ethanol.

Anal. Calcd. for $C_{19}H_{21}NO_4$: C, 69.7; H, 6.4; N, 4.3. Found: C, 70.3; H, 6.6; N, 4.1.

N-Benzyl-4-carboxy-2-pyrrolidone.—To a solution of 2.9 g. (0.02 mole) of monomethyl ester of itaconic acid, m.p. 70°, in 20 ml. of dioxane was added 2.1 g. (0.02 mole) of benzylamine. The solution was heated in an oil bath at 110–120° for 2 hr. The solvent was removed *in vacuo* and the pyrrolidone crystallized from water, m.p. and m.m.p.,[1] 144°.

Anal. Calcd. for $C_{12}H_{13}NO_3$: C, 65.7; H, 5.9; N, 6.4. Found: C, 66.1; H, 6.1; N, 6.6.

On using 2 moles of benzylamine, the benzylamine salt of the pyrrolidone was obtained; m.p. and m.m.p.,[1] 111°.

Benzylamide Derivative of N-Benzyl-4-carboxy-2-pyrrolidone.—To a solution of 5.2 g. (0.033 mole) of dimethyl itaconate in 20 ml. of dioxane was added 7.1 g. (0.066 mole) of benzylamine. The solution was heated in an oil bath at 110–120° for 2 hr. The solvent was removed *in vacuo*, and the product crystallized from water; yield, 3 g. (30%); m.p. 104°. It contained no methoxyl groups.

Anal. Calcd. for $C_{19}H_{20}N_2O_2$: N, 9.1. Found: N, 9.2.

(14) Y. Liwschitz and A. Zilkha, *J. Am. Chem. Soc.*, **76**, 3698 (1954); *J. Chem. Soc.*, 4394 (1954).

(15) A. Anschütz and W. Petri, *Ber.*, **13**, 1539 (1880).

Epoxide Studies. I. The Ring Opening of *cis*- and *trans*-N,N-Diethylphenylglycidamide

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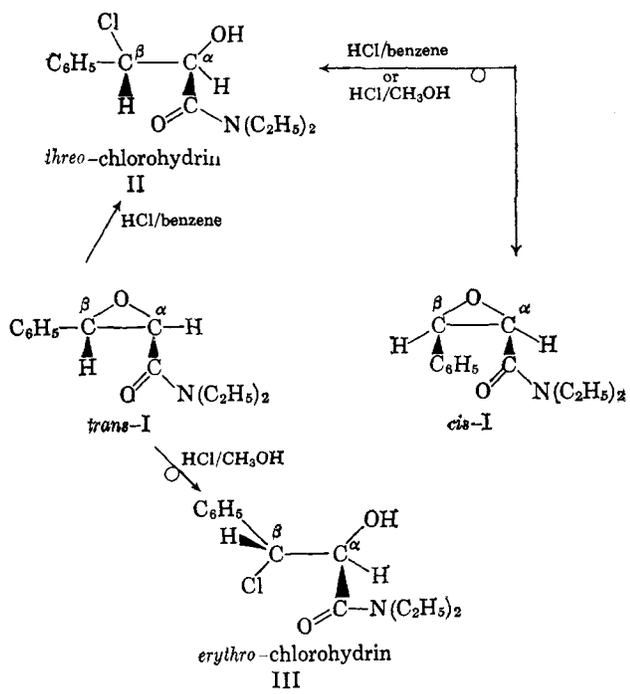
Treatment of *trans*-N,N-diethyl-3-phenylglycidamide (*trans*-I) with hydrogen chloride in benzene gave the *threo*-chlorohydrin II with retention of configuration whereas with hydrogen chloride in methanol, the *erythro*-chlorohydrin III was formed. *cis*-Glycidamide (*cis*-I) with either of these reagents afforded only II. In the presence of base, II gave mixtures of *cis–trans*-I and III gave only *trans*-I. The stereochemistry and mechanisms of these transformations are reported.

The opening of an epoxide ring by nucleophilic reagents has been regarded generally as a bimolecular nucleophilic displacement (SN2) on carbon proceeding with inversion of configuration.[1] For example, the reaction of *cis*- and *trans*-stilbene oxides with hydrogen

(1) (a) R. E. Parker and N. S. Isaacs, *Chem. Rev.*, **59**, 737–799 (1959); (b) S. Winstein and R. B. Henderson, "Heterocyclic Compounds," Vol. I, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1950, Chap. 1, pp. 27.

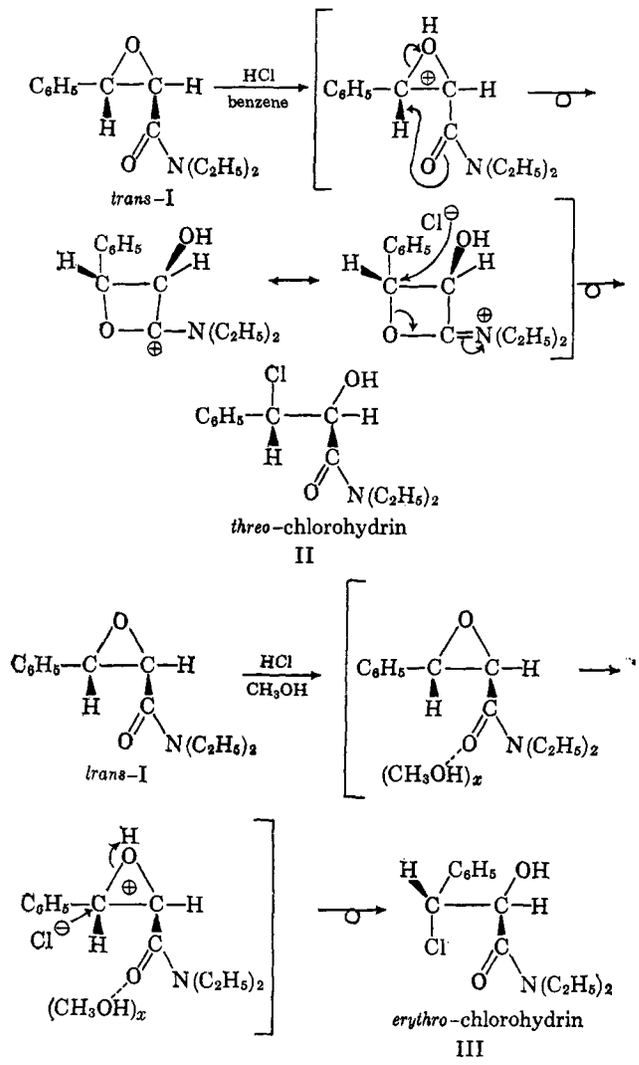
halides to form halohydrins[2] and the acid-catalyzed hydrolysis of *trans*-N,N-diethyl-3-phenylglycidamide to *erythro*-diol[3] both proceed with inversion of configuration. Certain epoxides, however, open with retention of configuration. The acid hydrolysis of *trans*-α-methylstilbene oxide to the *threo*-diol,[4] the conversion of *cis*- and *trans*-dypnone oxides to the *erythro*- and *threo*-chlorohydrins,[5] respectively, with hydrogen chloride in acetic acid or in ethanol, and the formation of the *threo*-diol from *trans*-3-phenylglycidic acid in dilute acid[6] proceed with retention of configuration.

This paper describes the stereochemistry of the ring opening of *cis*- and *trans*-N,N-diethyl-3-phenylglycidamides by hydrogen chloride in nonpolar (benzene) and polar (methanol) solvents. *trans*-N,N-Diethyl-3-phenylglycidamide (*trans*-I) with hydrogen chloride in benzene gave the *threo*-chlorohydrin II whereas with hydrogen chloride in methanol, the *erythro*-chlorohydrin III was formed. *cis*-N,N-Diethyl-3-phenylglycidamide (*cis*-I) with either of these reagents afforded only the *threo*-chlorohydrin II.[7] The *erythro* configuration for chlorohydrin III (m.p. 119–120°) was established by its conversion[3,8] to *trans*-I with base (98% yield).



The chlorohydrin II (m.p. 87–88°) obtained from *trans*-I was assigned the *threo* configuration since it was identical with the ring-opening product from *cis*-I and its infrared spectrum was identical with that of the *erythro* isomer III.

The observed retention of configuration in the formation of *threo*-chlorohydrin II from *trans*-I in benzene could be explained on the basis of participation of the neighboring amido group.[5,9] The net result would involve two inversions at the β-carbon atom or retention of configuration. In methanol, participation of the amido group is inhibited by hydrogen bonding (solvation) of the carbonyl group with the solvent. Consequently, the chlorohydrin III is formed with inversion of configuration. The clean inversion of configuration in the formation of the *threo*-chlorohydrin II from *cis*-I in benzene could be interpreted as the result of steric interaction. The effects attributable to the participation of the neighboring amido group could be eliminated or greatly reduced when phenyl and amido groups are *cis*.[10]



It is noteworthy that in the presence of base which brings about complete conversion of *erythro*-chlorohydrin III to *trans*-I, the *threo*-chlorohydrin II yields a mixture containing *cis*–*trans*-I in 60 and 40%, respectively.[11] Since *cis*-I does not undergo epimerization under the experimental conditions, the observed differences in intramolecular SN2 reactions of the diastereoisomeric chlorohydrins II and III are undoubtedly due to steric effects. With the aid of Newman projections, the formation of *trans*-I from *erythro*-chlorohydrin III would involve the more favored conformer in the transition state in which the two bulky groups (phenyl and diethylamido) are *trans* to each other. On the other hand, ring closure of *threo*-chloro-

(2) (a) D. Reulos, *Compt. rend.*, **216**, 774 (1943); (b) D. Reulos and C. Collin, *ibid.*, **218**, 795 (1944).

(3) C. C. Tung, A. J. Speziale, and H. W. Frazier, *J. Org. Chem.*, **28**, 1514 (1963).

(4) J. H. Brewster, *J. Am. Chem. Soc.*, **78**, 4061 (1956).

(5) H. H. Wasserman and N. E. Aubrey, *ibid.*, **78**, 1726 (1956).

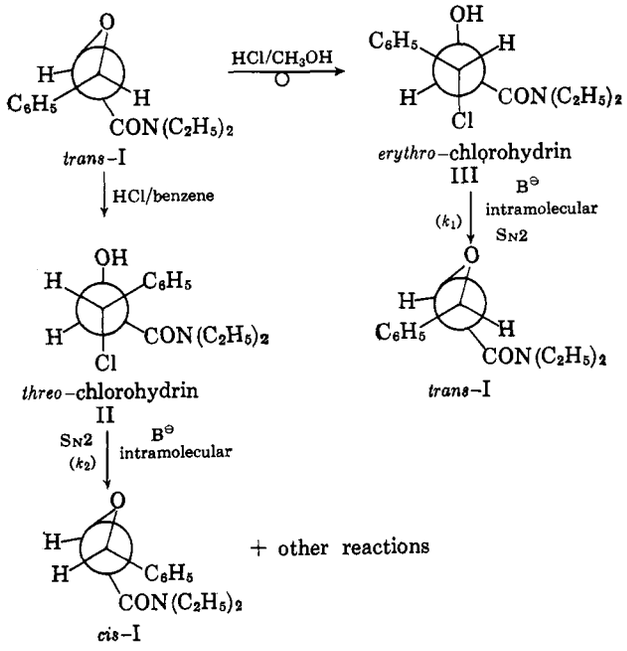
(6) J. Böeseken, *Rec. trav. chim.*, **41**, 199 (1922).

(7) The chlorohydrins are assigned the α-hydroxy-β-chloro structures; see ref. 3.

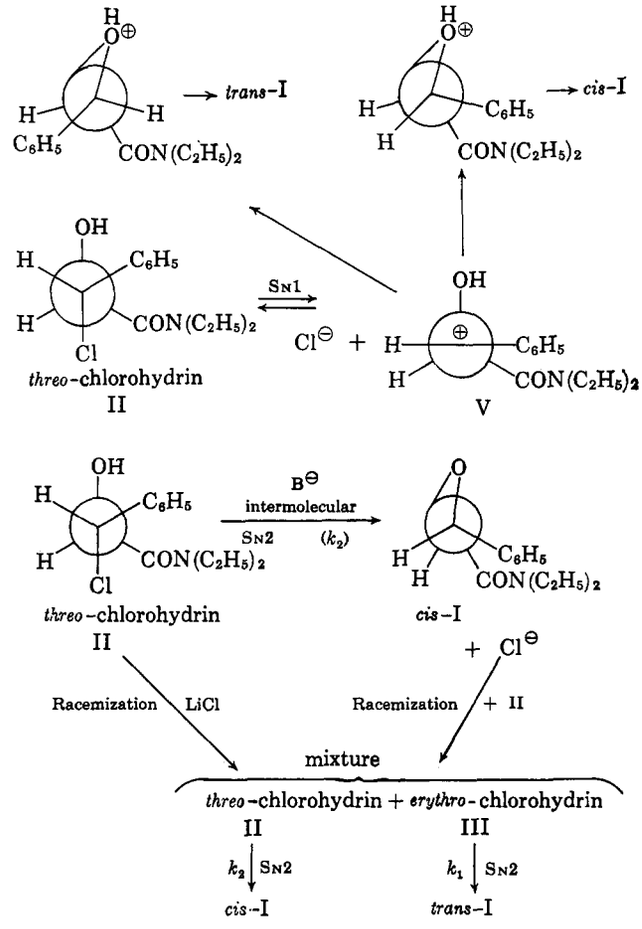
(8) (a) S. Winstein and H. J. Lucas, *J. Am. Chem. Soc.*, **61**, 1576 (1939); (b) H. J. Lucas and C. W. Gould, Jr., *ibid.*, **63**, 2541 (1941); (c) S. J. Cristol and W. P. Norris, *ibid.*, **75**, 632 (1953).

(9) S. Winstein and R. B. Henderson, *ibid.*, **65**, 2196 (1943).

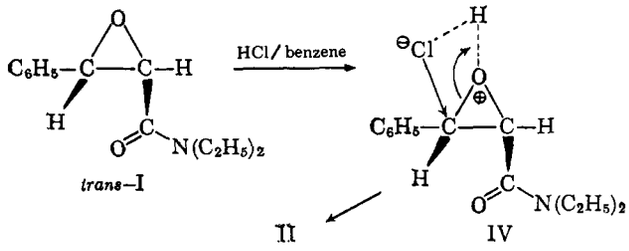
hydrin II to *cis*-I involves the eclipsing of two bulky groups. Because of this unfavorable conformation in the transition state for *threo*-chlorohydrin II, other reactions take place which lead to *trans*-I.



As a result of the relatively greater steric strain in the ring closure of the *threo*-chlorohydrin II, the rate of the intramolecular SN2 displacement of II to *cis*-I is undoubtedly slower than that of III to *trans*-I ($k_2 < k_1$). Racemization of II to form a mixture of the diastereoisomeric chlorohydrin II and III could, therefore, take place by chloride ion liberated from the ring closure. Consequently II would give a mixture of *cis*–*trans*-I. As a test of this mechanism, the reaction of the *threo*-chlorohydrin II with base was repeated, in the presence of lithium chloride. Since the rate of racemization is proportional to the concentration of the halide ion,[12] the ratio of *trans*-I–*cis*-I should be increased.[13] Indeed, from this experiment, we obtained a higher ratio of *trans*-I–*cis*-I (75:25)[14] as compared with the ratio of 40:60 in the absence of the lithium chloride.



(10) A less attractive interpretation for the observed retention of configuration for *trans*-I in benzene could be depicted as an internal displacement mechanism (SNi) [see W. A. Cowdry, E. D. Hughes, C. K. Ingold, S. Masterman, and A. D. Scott, *J. Chem. Soc.*, 1252 (1937), and D. J. Cram, *J. Am. Chem. Soc.*, **75**, 332 (1953)]. The ion pair IV would cleave so that chloride ion would attack the β-carbon atom from the same side as that undergoing C–O fission to give II. The observed inversion of configuration



in methanol could then be due to the shielding of the solvated ion pair and thereby facilitating the SN2 displacement at the β-position. A bimolecular nucleophilic displacement with inversion would, therefore, be favored. However, the previous interpretation appears untenable since the corresponding *cis*-I in both types of solvent gave only chlorohydrin II with inversion of configuration.

(11) The yields were calculated from n.m.r. spectrum. The reliability of n.m.r. spectra in quantitative determination of *cis*–*trans*-epoxy amides is demonstrated in a previous paper; see ref. 3.

(12) B. Holmberg, *J. prakt. Chem.*, (2) **88**, 553 (1913).

(13) For the racemization of alkyl halides by halide ion see E. D. Hughes, F. Jalinsberger, S. Masterman, B. Topley, and J. Weiss, *J. Chem. Soc.*, 1525 (1935).

Experimental

***threo*-N,N-Diethyl-3-chloro-2-hydroxy-3-phenylpropioamide (*threo*-Chlorohydrin II from *trans*-I).**—To a solution of 2.0 g. (0.075 mole) of dried (hydrogen sulfate) hydrogen chloride in 95 g. of benzene (dried over sodium) was added 4.24 g. (0.019 mole) of *trans*-N,N-diethyl-3-phenylglycidamide.[3] The solution was stirred at 25° for 1 hr. The solution was evaporated to dryness at room temperature under reduced pressure. The crude product was recrystallized from heptane–benzene to give 4.76 g. (97% yield) of colorless solid, m.p. 87–88°.

Anal. Calcd. for $C_{13}H_{18}ClNO_2$: C, 61.20; H, 7.12; N, 5.47; Cl, 13.89; mol. wt., 255. Found: C, 61.14; H, 7.02; N, 5.44; Cl, 14.23; mol. wt.,[15] 259.

***erythro*-N,N-Diethyl-3-chloro-2-hydroxy-3-phenylpropioamide (*erythro*-Chlorohydrin III from *trans*-I).**—To a stirred solution at 2° containing 31 g. of 22% by weight of hydrogen chloride (0.187 mole) in methanol was added dropwise a solution of 8.18 g. (0.0373 mole) of *trans*-N,N-diethyl-3-phenylglycidamide in 20 ml. of methanol. The solution was allowed to stand at room temperature for 1 hr. Removal of the solvent under vacuum left a light yellow viscous oil. The oil was taken up in chloroform, washed with dilute sodium bicarbonate solution, then with water, and dried over magnesium sulfate. The crude material after recrystallization from hexane gave 8.96 g. (94% yield) of colorless plates, m.p. 119–120°.

(14) A less favorable mechanism for the formation of *cis*–*trans*-I mixture from *threo*-chlorohydrin II would be as depicted. Although benzyl chloride will readily undergo SN1 reactions because of the resonance stabilization of the benzyl carbonium ion V, this reaction would be depressed by the presence of a common ion salt such as lithium chloride. Consequently, a decrease in the ratio of *trans*-I–*cis*-I should be observed. See L. C. Bateman, E. D. Hughes, and C. K. Ingold, *ibid.*, 1017 (1940).

(15) Molecular weight has determined by vapor pressure osmometer using benzene as the solvent.

Anal. Calcd. for $C_{13}H_{18}ClNO_2$: C, 61.20; H, 7.12; N, 5.47; Cl, 13.89; mol. wt., 255. Found: C, 61.34; H, 7.27; N, 5.47; Cl, 14.02; mol. wt.,[15] 242.

Preparation of *threo*-Chlorohydrin II from *cis*-I.—By following the same procedure as described for *trans*-I, *cis*-I gave 84% yield of *threo*-chlorohydrin II, m.p. 87–88°, from hydrogen chloride in benzene solution and 76% yield of II, m.p. 87–88°, from hydrogen chloride in methanol. A mixture melting point of these chlorohydrins with that from *trans*-I gave no depression and their infrared spectra were identical. Their elemental analyses and molecular weights were in agreement with those calculated for the desired product.

Treatment of *erythro*-Chlorohydrin III with Base.—A solution of 0.20 g. (0.0037 mole) of sodium methoxide in 10 ml. of methanol was added over a period of 10 min. to a solution of 0.94 g. (0.0037 mole) of *erythro*-chlorohydrin III in 11 ml. of 95% ethanol at 0°. After standing at room temperature for 20 min., the solvent was evaporated *in vacuo* at room temperature. The solid was stirred with water and taken up in ether. The ether solution was washed with water and dried over magnesium sulfate. Removal of ether afforded 0.84 g. of a colorless solid which was chromatographed on alumina. Elution with chloroform–benzene gave 0.80 g. (98% yield) of *trans*-I, m.p. 87–88°. The infrared and n.m.r. spectra were identical with that of *trans*-I.[3]

Treatment of *threo*-Chlorohydrin II with Base.—The same procedure as described for *erythro*-chlorohydrin III with base was followed. There was obtained 0.74 g. (91% yield) of viscous oil which solidified slowly on standing. The n.m.r. spectrum[3] indicated this product to consist solely of 60% *cis*-I and 40% of *trans*-I.[11]

Treatment of *threo*-Chlorohydrin II with Base in the Presence of Lithium Chloride.—A solution of 0.1150 g. (0.00212 mole) of sodium methoxide in 5 ml. of 95% ethanol was added to a solution of 0.5411 g. (0.00212 mole) of *threo*-chlorohydrin II and 0.1217 g. (0.00287 mole) of lithium chloride in 20 ml. of 95% ethanol. The solution was allowed to stand at room temperature for 16 hr. After the work-up as described in the previous experiments, there remained 0.3953 g. (85% yield) of solid. The n.m.r. spectrum[3] showed this material to contain only 25% of *cis*-I and 75% of *trans*-I.[11]

Epimerization Study of *cis*-I with Base.—When the *cis*-I was treated with sodium methoxide in ethanol solution at room temperature, the n.m.r. spectrum and melting point of the product were identical with the starting *cis*-I.

Nitriles and Amidines of Optically Active Acylamino Acids and Peptides

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Nitriles of acylated amino acids and peptides were made by treatment of the corresponding amides with pyridine and phosphorus oxychloride. The optical activity was retained. The amidines were made by conversion of the nitriles to iminoethers, which then were converted to amidines. In this way acetyl-L-phenylalanine nitrile, α-acetyl-ε-tosyllysine nitrile, N-benzoyl-L-phenylalanylserine nitrile, N-benzoyl-L-phenylalanyl-O-benzylserine nitrile, N-acetyl-L-phenylalanineamidine, the corresponding N-benzylamidine, α-acetyl-ε-tosyllysineamidine, and the corresponding N-benzylamidine were prepared. The conversion of the dipeptide nitriles to their amidines was largely unsuccessful because of an intramolecular decomposition which gave N-benzoylserineamidine from N-benzoylphenylalanylserine nitrile.

To understand the fundamental mechanism of action of certain enzymes, Woolley, *et al.*,[1] proposed that polypeptides of two new and unusual amino acids should be synthesized. One of these amino acids, which may be given the trivial name of acetylphenylalanohistidine (Fig. 1), was expected, when polymerized, to exhibit the specific enzymic activity of chymotrypsin, and the other, acetyllysohistidine, was expected to have the specific activity of trypsin. The synthesis of these compounds, according to either of the two routes previously explored,[1] would require the preparation of amidines and N-benzylamidines of acetylated α-amino acids. The purpose of the present paper is to describe the synthesis of such amidines in optically active condition. Such optical activity was necessary for the purpose in hand. In addition, a third route leading toward phenylalanohistidine has been explored. It would begin with the amidine of the dipeptide, benzoylphenylalanylserine, which would then be caused to cyclize to a 4-aminoimidazole according to the method of imidazole ring formation developed by Shaw and Woolley.[2] The peptide should thus yield an imidazole which, by removal of the 4-amino group and completion of the histidine side chain, should give the desired phenylalanohistidine derivative. The formation of dipeptide amidines was, therefore, investigated.

A convenient route to amidines starts with nitriles which are converted to imino ethers (imino esters) with alcohol and hydrogen chloride. The imino ethers are then treated with ammonia or other amines to yield the amidines. Racemic α-aminonitriles and their acyl derivatives have been known for a long time since they are intermediates in the Strecker synthesis of amino acids from aldehydes and ammonium cyanide. Because optically active amidines were needed for the present work, a route which could be expected to yield optically active compounds was sought. The dehydration of optically active amides to optically active nitriles was attempted. The usual methods for this reaction (phosphorus oxychloride, phosphorus pentoxide, toluenesulfonyl chloride) failed when applied to acetylphenylalanine amide or α-acetyl-ε-tosyllysine amide. However, the method of Delaby, *et al.*,[3] in which an amide is treated briefly in the cold with pyridine and phosphorus oxychloride, succeeded both for the amino acid derivatives as well as for the peptide derivatives.

The conversion of the nitriles to amidines proved to be difficult, but was accomplished finally when adequate methods for the separation of the final products were developed and when the lability to acid of the acetyl group attached to the α-amino group was appreciated. Thus, in the formation of the imino ethers it was necessary to avoid large excesses of hydrogen chloride. Similarly, in order to escape deacetylation, strong acids

(1) D. W. Woolley, J. W. B. Hershey, and I. H. Koehelik, *Proc. Natl. Acad. Sci. U. S.*, **48**, 709 (1962).

(2) E. Shaw and D. W. Woolley, *J. Biol. Chem.*, **181**, 89 (1949).

(3) R. Delaby, G. Tsatsas, and X. Lusinchi, *Bull. soc. chim. France*, 409 (1958).