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).

were not used during the isolation of the amidines. No effort was made to isolate the imino ethers, but, instead, they were converted directly to the amidines. These were obtained pure only by means of countercurrent distribution. The conventional methods of isolation failed, probably because of the difficulty with which these amidine hydrochlorides crystallized.

A curious rearrangement prevented the realization of the dipeptide amidines. When N-benzoylphenylalanylserine nitrile was subjected to the reactions for formation of the imino ether and amidine, a mixture was obtained. The only product which was isolated in pure condition was benzoylserine amidine. The phenylalanine residue had been extruded. It was thought that protection of the hydroxyl group of the serine residue might prevent this surprising reaction, but when N-benzoylphenylalanyl-O-benzylserine nitrile was treated similarly, the same type of rearrangement occurred. A fraction was obtained which yielded on hydrolysis only O-benzylserine and no phenylalanine. It was not possible to separate the desired dipeptide amidine from either reaction in analytically pure condition, although in both cases a separate fraction was obtained by countercurrent distribution, which on hydrolysis gave the expected two amino acids. The yields, however, were too small to justify pursuit of this route to the phenylalanohistidine compounds.

Experimental

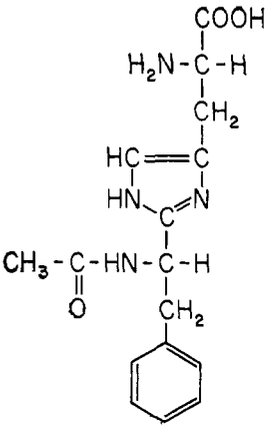
Methods.—All melting points were determined in capillary tubes. All evaporations were conducted under reduced pressure below 40°. All compounds were recrystallized to constant melting point. Countercurrent distributions were carried out in the apparatus of Craig. Paper electrophoresis was done at room temperature with paper strips 54 cm. long in 0.1 *M* pyridine acetate at pH 5.0 and with 800 v. across the length of the paper. When "dry" pyridine was required, it was distilled from sodium shortly before use. Chloroform was dried over calcium chloride.

N-Acetyl-L-phenylalanine Nitrile, or L-2-Acetamido-3-phenylpropionitrile.—A solution of 20.6 g. (100 mmoles) of N-acetyl-L-phenylalanine amide in 320 ml. of dry pyridine was cooled to −5°, stirred vigorously, and treated dropwise with 10 ml. (100 mmoles) of freshly distilled phosphorus oxychloride. The addition required 10 min. The mixture was held at 0° for an additional 10 min. and was then quickly concentrated below 40° to a sirup (less than 80 g.). Benzene (700 ml.) and water (200 ml.) were added, followed by enough concentrated hydrochloric acid to give a pH of 2–3 in the aqueous phase. The aqueous phase was separated and extracted twice more with benzene. The combined extracts were washed with 20 ml. of water which contained enough sodium bicarbonate to give a final pH of 7, dried with magnesium sulfate, and freed of solvent under reduced pressure. The residue was then recrystallized from hot benzene (50 ml.) by addition of hexane (125 ml.). The yield was 9.5 g.; m.p. 106–108°, unchanged by further crystallization; $[\alpha]^{21}D$ −10.2° (*c* 2.5, ethanol).

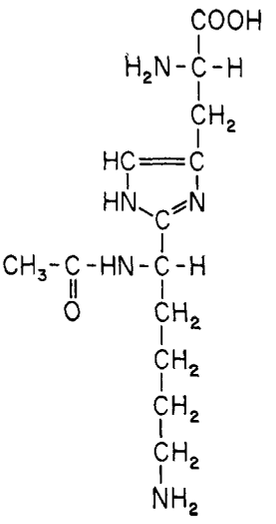
Anal. Calcd. for $C_{11}H_{12}N_2O$: C, 70.2; H, 6.4; N, 14.9. Found: C, 70.0; H, 6.6; N, 15.1.

Although N-acetyl-L-phenylalanine amide has been described as being prepared *via* the ester, it was found advantageous to make it from acetylphenylalanine by the mixed anhydride method with triethylamine, ethyl chloroformate, and aqueous ammonia.

N-Acetyl-L-phenylalanineamidine Hydrochloride.—Acetyl-L-phenylalanine nitrile (5.64 g., 30 mmoles) dissolved in 30 ml. of dry chloroform was cooled to 4° and treated with 5.4 ml. of absolute ethanol which was 6.6 *M* with respect to dry hydrogen chloride (20% excess hydrogen chloride). The solution was held at 4° overnight and then at 25° for 2 hr. At this point the imino ether hydrochloride had separated either as an oil or as crystals. The solvents were removed under reduced pressure at 25° and the residue was dissolved in 10 ml. of absolute ethanol at 0°.



Acetylphenylalanohistidine



Acetyllysohistidine

Figure 1

A saturated solution of dry ammonia in absolute ethanol (20 ml.) was added and the mixture was held in a tightly stoppered flask for 4 days at 25°. The solvents were removed under reduced pressure and the residue was dissolved in the system methanol–chloroform–water, 2:2:1; the top layer was adjusted to pH 3 with hydrochloric acid; and a countercurrent distribution was performed. The positions of the peaks were located by measurement of absorption at 260 mμ. Unchanged nitrile (1.11 g.) was found in tubes 0 to 30 after 160 transfers and could be recovered. The desired amidine exhibited its peak in tube 130. The contents of the tubes which contained the amidine were combined and a small amount of ammonium chloride was removed by evaporation of the solvents and extraction of the residue with absolute ethanol, in which the amidine hydrochloride was very soluble. The yield was 2.2–3.3 g.

The crystallization of this amidine hydrochloride was extremely difficult. Usually it was obtained an as amorphous solid, but it could be crystallized by rubbing with chloroform. It was rather hygroscopic. It gave no ninhydrin test when it was pure but, if any deacetylated amidine was present, it gave a positive test with ninhydrin.

The pure amidine hydrochloride showed only slight optical activity, $[\alpha]^{26}D$ +2.7° (*c* 3.0, ethanol). The low optical activity was not the result of racemization of the optical center because hydrolysis for 24 hr. with refluxing 6 *N* hydrochloric acid yielded L-phenylalanine with the correct rotation. The amidine was unusually difficult to hydrolyze completely with acid. Conditions which caused complete liberation of phenylalanine from acetylphenylalanine amide (6 *N* hydrochloric acid, 20 hr. at 100°) still left detectable amounts of the deacetylated amidine (*i.e.*, phenylalanineamidine).

If the holding of the imino ether reaction at 25° for 2 hr. was omitted, the yield was considerably reduced, but, if the conditions were made more vigorous than those given before, some deacetylation occurred at the imino ether stage. The hydrochloride was so hygroscopic that its melting point (82°) was not a useful criterion of purity. The compound did not form a water-insoluble picrate or flavianate.

Anal. Calcd. for $C_{11}H_{16}ClN_3O$: C, 54.7; H, 6.6; N, 17.4. Found: C, 54.0; H, 6.6; N, 17.4.

In order to obtain analytical values for carbon approaching the theoretical, it was necessary to burn the compound slowly. With the usual conditions for burning, values for C were always low (52 to 53% C).

Acetylphenylalanine-N-benzylamidine Hydrochloride.—The imino ether hydrochloride was prepared from acetyl-L-phenylalanine nitrile (1.88 g., 10 mmoles) exactly as described for the preceding experiment, but, instead of an alcoholic solution of ammonia, a solution of 1.25 ml. (11.5 mmoles) of benzylamine in 5 ml. of absolute ethanol was used. It was important to avoid the use of a larger excess of benzylamine. The subsequent operations were then the same as for the unbenzylated amidine except that the countercurrent system was chloroform–methanol–water, 2:1:1. In the countercurrent separation, the benzylamidine showed a peak in tube 74 of 97 transfers. The benzylamidine

hydrochloride was difficult to crystallize, but, by careful addition of ether to a dry, alcoholic solution, crystals which melted at 165° could sometimes be obtained. It did, however, form a picrate which was slightly soluble in water and well crystallized. When recrystallized the picrate melted at 135–137°. The yield of hydrochloride was 1.2 g.

Anal. Calcd. for $C_{18}H_{22}ClN_3O$: C, 65.1; H, 6.6; N, 12.7. Found: C, 64.2; H, 6.8; N, 12.6.

Calcd. for picrate, $C_{24}H_{24}N_6O_8$: C, 55.0; H, 4.6; N, 16.0. Found: C, 54.7; H, 4.7; N, 15.9.

It was found quite impossible to isolate either the benzylated or unbenzylated amidine without the use of countercurrent distribution.

Hydrolysis for 24 hr. in refluxing 6 *N* hydrochloric acid yielded phenylalanine and benzylamine as revealed by paper electrophoresis. If the hydrolysis was for less than 24 hr., a third spot always appeared on the papers. This was due to phenylalanine-benzylamidine which moved 12 cm. at pH 5.0 when histidine moved 13.8 cm. and benzylamine 17.5 cm.

Phenylalanine-N-benzylamidine Dihydrochloride.—When a solution of 1 g. of acetylphenylalanine-N-benzylamidine hydrochloride in 100 ml. of 0.05 *N* hydrochloric acid was concentrated under reduced pressure at 40° to a sirup and this residue was triturated with absolute ethanol (10 ml.), a crystalline solid remained undissolved. This was recrystallized from water by addition of ethanol to give L-phenylalanine-N-benzylamidine dihydrochloride (111 mg.), m.p. 215°.

Anal. Calcd. for $C_{16}H_{21}Cl_2N_3$: C, 58.9; H, 6.4. Found: C, 59.1; H, 6.6.

Hydrolysis of this compound, and electrophoresis of the hydrolysate gave benzylamine and phenylalanine. The intact amidine formed a purple color with ninhydrin and moved 12 cm. at pH 5.0 when histidine alongside moved 13.8 cm.

α-Acetyl-ε-tosyl-L-lysine—ε-Tosyl-L-lysine (25 g., 83 mmoles) was acetylated with 9.2 ml. (98 mmoles) of acetic anhydride by the usual Schotten–Baumann procedure for the acylation of amino acids. The product was recrystallized from water; 27.4 g., m.p. 145–146°; $[\alpha]^{23}D$ +6.1° (*c* 3.0, ethanol).

Anal. Calcd. for $C_{15}H_{22}N_2O_5S$: C, 52.6; H, 6.5; N, 8.2. Found: C, 52.5; H, 6.4; N, 8.2.

α-Acetyl-ε-tosyl-DL-lysine Amide.—A solution of α-acetyl-ε-tosyl-L-lysine (21.1 g., 61.7 mmoles) and triethylamine (17.3 ml., 123.4 mmoles) in 500 ml. of dry tetrahydrofuran was cooled to −10°. Ethyl chloroformate (6.5 ml., 67.9 mmoles) was added dropwise during 5 min. with vigorous mechanical stirring. The solution was stirred an additional 10 min. and then 13 ml. of aqueous concentrated ammonium hydroxide was added all at once. The cooling bath was removed and the contents stirred 30 min. Water (200 ml.) was added and the solution was concentrated to about 150 ml. and stored overnight at 4°. The white precipitate was filtered off and recrystallized from water; 18.7 g., m.p. 167–168°.

Anal. Calcd. for $C_{15}H_{23}N_3O_4S$: C, 52.7; H, 6.8; N, 12.3. Found: C, 52.5; H, 6.8; N, 12.2.

The lysine underwent racemization during the formation of the amide. This was demonstrated by hydrolysis (refluxing 6 *N* hydrochloric acid for 3 hr.) to ε-tosyllysine, which was isolated. It was found to have no rotation, whereas material similarly isolated from the hydrolysis of α-acetyl-ε-tosyllysine (the starting material of the synthesis) had $[\alpha]^{22}D$ +13.1° (*c* 3.0, 2 *N* hydrochloric acid) (lit.[4] $[\alpha]^{22}D$ +13.6°).

Attempts to synthesize the amide by treatment of the corresponding ester with alcoholic ammonia were unsuccessful.

α-Acetyl-ε-tosyl-DL-lysine Nitrile.—α-Acetyl-ε-tosyl-DL-lysine amide (10.0 g., 29.3 mmoles) in 200 ml. of dry pyridine was treated dropwise with phosphorus oxychloride (2.69 ml., 29.3 mmoles) at −5° and worked up as in the case of acetylphenylalanine nitrile, except that ethyl acetate instead of benzene was used for partitioning. The crude product, 7.5 g., was finally purified by countercurrent distribution (60 transfers) in the system ethanol 460–ethyl acetate 675–hexane 225–water 440. The nitrile peak was in tube 32. A small amount of amide with a peak in tube 8 was thus removed. The nitrile could not be crystallized. Infrared spectroscopy showed an absorption peak for nitriles at 2235 $cm.^{-1}$.

Anal. Calcd. for $C_{15}H_{21}N_3O_3S$: C, 55.7; H, 6.6; N, 13.0. Found: C, 55.4; H, 6.7; N, 12.5.

(4) R. Roeske, F. H. C. Stewart, R. J. Stedman, and V. du Vigneaud, *J. Am. Chem. Soc.*, **78**, 5883 (1956).

α-Acetyl-ε-tosyl-DL-lysineamidine Hydrochloride.—α-Acetyl-ε-tosyl-DL-lysine nitrile (7.0 g., 21.5 mmoles) was dissolved in 1.5 ml. of ethanol (25 mmoles) and 50 ml. of dry chloroform. Dry hydrogen chloride was bubbled through the solution for 20 min. at 0°. The flask was tightly stoppered and placed at 4° overnight. The solvents were removed under reduced pressure and the residue was dissolved in cold ethanol saturated with ammonia (150 ml.). The solution was held at room temperature in a tightly stoppered flask for 3 days. The solvents were removed under reduced pressure and the residue was dissolved in the solvent system chloroform–methanol–0.1 *N* hydrochloric acid, 3:2:2, acidified to about pH 1 and separated by countercurrent distribution (100 transfers). The amidine hydrochloride was in tubes 80 to 95, whereas unchanged nitrile was maximal in tube 15 and a small amount of amide was maximal in tube 45. The amidine fraction was brought to pH 5 with ammonium hydroxide, evaporated, and the residue freed of ammonium chloride by fractional precipitation with ether from absolute ethanol. By careful addition of more ether, crystals of the amidine hydrochloride were obtained; m.p. 171–172°.

Anal. Calcd. for $C_{15}H_{25}ClN_4O_3S$: C, 47.8; H, 6.6; N, 14.9. Found: C, 47.3; H, 6.6; N, 14.5.

The amidine picrate was prepared and crystallized from water; m.p. 172–174°.

Anal. Calcd. for $C_{21}H_{27}N_7O_{10}S$: N, 17.2. Found: N, 17.2.

α-Acetyl-ε-tosyl-DL-lysine-N-benzylamidine Acetate.—The α-acetyl-ε-tosyl-DL-lysine nitrile (6.37 g., 19.7 mmoles) was converted to the imino ether hydrochloride, as in the preceeding example, and was caused to react with benzylamine (3.26 ml., 30 mmoles) in absolute ethanol for 3 days at room temperature. Evaporation of the solvents and countercurrent distribution (96 transfers) in 1-butanol–water–acetic acid, 15:18:2, gave the benzylamidine as the acetate with a peak in tube 84. Evaporation of the solvents gave the oily salt which was purified further by precipitation from alcohol with ether, but which was never crystallized.

Anal. Calcd. for $C_{22}H_{30}N_4O_3S \cdot C_2H_4O_2$: C, 58.7; H, 7.0; N, 11.4. Found: C, 58.4; H, 6.5; N, 11.3.

N-Benzoyl-L-phenylalanylserine Ethyl Ester.—DL-Serine ethyl ester hydrochloride (1.7 g., 10 mmoles) was suspended in 20 ml. of methylene chloride and treated with 1.41 ml. of triethylamine. The suspension was stirred for 5 min., filtered, and the filtrate was treated with a solution of 2.1 g. (10 mmoles) of dicyclohexylcarbodiimide in 10 ml. of methylene chloride, and immediately thereafter with 2.7 g. of benzoyl-L-phenylalanine in 10 ml. of the same solvent. The mixture was held at room temperature overnight, treated with 0.3 ml. of acetic acid for 1 hr., and filtered. The filtrate was extracted with aqueous *N* hydrochloric acid and then with sodium bicarbonate, and the solvent was evaporated. A small amount of dicyclohexylurea was removed by solution of the dipeptide ester in ethyl acetate and filtration. The ester was then crystallized from ethyl acetate–benzene to yield 1.2 g. of the pure compound. It was not determined whether both diastereoisomers were present or whether resolution of the serine residue had occurred during the recrystallization. The use envisioned for the dipeptide did not require optically active serine. This use was the conversion to 2-(α-benzamido-β-phenylethyl)-4-amino-5-hydroxymethylimidazole in which the asymmetric center of the serine would disappear. The dipeptide ester melted at 142–143°.

Anal. Calcd. for $C_{21}H_{24}N_2O_5$: C, 65.6; H, 6.3; N, 7.3. Found: C, 65.6; H, 6.3; N, 7.4.

N-Benzoyl-L-phenylalanylserine Amide. (A) From the Ester.—N-Benzoyl-L-phenylalanylserine ethyl ester (1.2 g.) was dissolved in 50 ml. of absolute ethanol and the solution was saturated with dry ammonia. After 4 days at room temperature, the reaction mixture was concentrated under reduced pressure to dryness and the residue was recrystallized from ethyl acetate by addition of benzene. The yield was 1.0 g.; m.p. 176°, unchanged by further recrystallization.

Anal. Calcd. for $C_{19}H_{21}N_3O_4$: C, 64.2; H, 5.9; N, 11.8. Found: C, 64.6; H, 5.9; N, 11.5.

(B) From Serine Amide.—A suspension of 3.13 g. (20 mmoles) of DL-serine amide hydrochloride in 25 ml. of methylene chloride was stirred and treated with 2.82 ml. (20 mmoles) of triethylamine. Immediately thereafter, the resulting suspension was treated with a solution of 4.2 g. (20 mmoles) of dicyclohexylcarbodiimide in 20 ml. of methylene chloride, and then with 5.4 g. (20 mmoles) of N-benzoyl-L-phenylalanine in 30 ml. of the same solvent. The mixture was stirred at room temperature

overnight and then worked up as described before for the corresponding ester. The resulting dipeptide amide was recrystallized as described in A and was found to melt at 176°.

N-Benzoyl-L-phenylalanyl-O-benzylserine Ethyl Ester.—DL-O-Benzylserine (9.75 g., 50 mmoles) was converted to its ethyl ester hydrochloride by treatment first with refluxing absolute ethanol containing 2 equivalents of dry hydrogen chloride and then by repeated treatment with refluxing absolute ethanol in the customary fashion for esterifications. The ester hydrochloride was dried thoroughly *in vacuo* and then was dissolved in methylene chloride (50 ml.). Condensation was then carried out with benzoyl-L-phenylalanine (13.5 g., 50 mmoles) by addition of triethylamine (7.05 ml., 50 mmoles) and dicyclohexylcarbodiimide (10.3 g., 50 mmoles) exactly as was described in the preceding examples. The product was recrystallized from ethyl acetate–benzene; m.p. 120–121°.

Anal. Calcd. for $C_{28}H_{30}N_2O_5$: C, 70.9; H, 6.3; N, 5.9. Found: C, 70.9; H, 6.4; N, 5.7.

N-Benzoyl-L-phenylalanyl-O-benzylserine.—The ethyl ester of the preceding section (21.6 g.) was dissolved in 200 ml. of ethanol and treated with 60 ml. of *N* sodium hydroxide for 2 hr. Acidification with hydrochloric acid, evaporation of the ethanol, and partition between benzene and water at pH 8 gave the sodium salt of the desired acid in the aqueous phase. Acidification yielded an oil which was crystallized from benzene to give 17.6 g., m.p. 162–163°.

Anal. Calcd. for $C_{26}H_{26}N_2O_5$: C, 70.0; H, 5.8; N, 6.3. Found: C, 69.8; H, 5.7; N, 6.1.

N-Benzoyl-L-phenylalanyl-O-benzylserine Amide.—In contrast to the behavior of benzoylphenylalanylserine ethyl ester, the corresponding O-benzylserine ester failed to yield the amide when treated either with alcoholic ammonia at room temperature, or with liquid ammonia in a bomb at room temperature. Unchanged ester was always recovered. Consequently, the desired amide was prepared from the dipeptide acid by way of the mixed anhydride.

Benzoyl-L-phenylalanyl-O-benzylserine (17.6 g., 40 mmoles) in 100 ml. of tetrahydrofuran and 5.61 ml. (40 mmoles) of triethylamine was stirred and cooled to −5° and treated dropwise with 4.3 ml. (45 mmoles) of ethyl chloroformate. Ten minutes after the end of the addition, concentrated aqueous ammonia (11 ml.) was added quickly in one portion. The mixture was stirred for 30 min., diluted with 250 ml. of water, and concentrated under reduced pressure to 75 ml. The solution was then extracted four times with ethyl acetate at pH 7.0 and the combined extracts were evaporated. The residue was recrystallized from benzene to yield 8.8 g., m.p. 157–160°.

Anal. Calcd. for $C_{26}H_{27}N_3O_4$: C, 70.1; H, 6.1; N, 9.4. Found: C, 70.2; H, 6.0; N, 9.3.

N-Benzoyl-L-phenylalanyl-O-acetylserine Nitrile.—Benzoyl-L-phenylalanylserine amide (2.24 g., 6.3 mmoles) was dissolved in 15 ml. of dry pyridine, and the solution was cooled to −5°, stirred, and treated first with 0.5 ml. (7.0 mmoles) of acetyl chloride and then with 0.64 ml. (6.3 mmoles) of phosphorus oxychloride. The reaction mixture was worked up in the way described for acetylphenylalanine nitrile and the product was recrystallized from benzene; 400 mg.; m.p. 169–170°.

Anal. Calcd. for $C_{21}H_{21}N_3O_4$: C, 66.5; H, 5.6; N, 11.1. Found: C, 66.1; H, 5.7; N, 10.8.

N-Benzoyl-L-phenylalanyl-O-benzylserine Nitrile.—Treatment of benzoylphenylalanyl-O-benzylserine amide with pyridine and phosphorus oxychloride in the manner described for acetylphenylalanine amide gave the desired nitrile which was crystallized from benzene. The yield was 2.8 g. from 8.3 g. of amide, m.p. 142–144°.

Anal. Calcd. for $C_{26}H_{25}N_3O_3$: N, 9.8. Found: N, 9.6.

Formation of Benzoylserineamidine in the Attempted Conversion of Benzoylphenylalanylserine Nitrile to the Dipeptide Amidine.—Benzoylphenylalanylserine nitrile (1.87 g.) was converted to the imino ether in chloroform solution (15 ml.) by addition of 0.71 ml. of 8.4 *M* ethanolic hydrogen chloride in the manner described for the corresponding reaction with acetylphenylalanine nitrile. The reaction product was then treated at room temperature for 4 days with ethanolic ammonia. The product of this reaction was then separated countercurrently through 96 transfers in the solvent system composed of chloroform–methanol–water, 2:1:1. Peaks detectable by ultraviolet absorption at 260 mμ were found in the starting tubes (unchanged nitrile), in tube 50, and in the final tubes. The peak in tube 50 probably contained the desired dipeptide amidine hydrochloride, because it yielded serine and phenylalanine when hydrolyzed, but an analytically pure compound was not obtained from it. The material in tubes 81–99 was redistributed through 68 transfers in the solvent system, chloroform–methanol–water, 2:2:1. The peak of ultraviolet absorption was in tube 53. The material recovered from this peak by evaporation of the solvents was a glassy substance (200 mg.) which proved to be benzoylserineamidine hydrochloride. When hydrolyzed and chromatographed on paper, it gave serine as the only amino acid.

Anal. Calcd. for $C_{10}H_{14}ClN_3O_2$: C, 49.3; H, 5.7; N, 17.2. Found: C, 49.8; H, 5.7; N, 17.1.

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Ultraviolet Spectra and Polarographic Reduction Potentials of Some Cinnamic Acids[1]

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The effect of *para* and *ortho* substitutents on the ultraviolet spectra and half-wave reduction potentials of *trans*-cinnamic acid is interpreted in terms of their electronic and, in the case of *ortho*-substitution, steric effects. Only qualitative relations exist between the spectroscopic and polarographic data.

The effect of substituents on the dissociation constants[2a] of *trans*-cinnamic acid and the rates of hydrolysis of its ethyl esters[2b] have been discussed. However, no detailed study has been published concerning the effect of the substituents on the ultraviolet spectra or polarographic reduction potentials of these acids. (Such limited data as are available on the ultraviolet spectra of simple derivatives is indicated in the footnotes to Table I[3]).

A series of *trans*-cinnamic acids was prepared (see Table II) by standard methods and their ultraviolet spectra determined in 95% ethanol. These acids showed two regions of high intensity absorption (Table I), at 215–230 mμ and 270–320 mμ. The first region usually possessed two maxima of about equal intensity and the wave lengths and intensities varied little with

(1) Contribution number 130 from the Instituto de Química de la Universidad Nacional Autonoma de México.

(2) (a) *Cf.* M. Charton and H. Meislich, *J. Am. Chem. Soc.*, **80**, 5940 (1959); (b) B. Jones and J. G. Watkinson, *J. Chem. Soc.*, 4064 (1958).

(3) *Cf.* A. Mangini and F. Montanari, *Boll. sci. fac. chim. ind., Bologna* **12**, 166 (1954).