$-970°$, $[\phi]_{445}$ $-1110°$, $[\phi]_{408}$ $-470°$, $[\phi]_{354}$ $-3120°$; (c 0.011) $[\phi]_{354}$ $-3100°$, $[\phi]_{350}$ $-5400°$, $[\phi]_{305}$ $+11{,}000°$, $[\phi]_{270}$ $-3500°$.

Anal. Calcd. for $C_{15}H_{14}ClNO$: C, 69.36; H, 5.43. Found: C, 69.41; H, 5.69.

(*S*)-(+)-**N-5-Chlorosalicylidene-α-benzylethylamine** (**Vb**).—Addition of (*S*)-(+)-α-benzylethylamine to a 1% excess of 5-chlorosalicylaldehyde in methanol gave Vb (63% yield), microscopic light yellow needles, m.p. 75–76° (heptane), $[\alpha]^{22}D$ $+264°$, $[\phi]^{22}D$ $+737°$ (c 1.0, absolute ethanol); R.D. in 95% ethanol, 26°: (c 1.0) $[\phi]_{600}$ $+829°$, $[\phi]_{589}$ $+869°$, $[\phi]_{470}$ $+1960°$; (c 0.051) $[\phi]_{470}$ $+2000°$, $[\phi]_{447}$ $+2300°$, $[\phi]_{403}$ $+850°$, $[\phi]_{360}$ $+4300°$; (c 0.021) $[\phi]_{360}$ $+4000°$, $[\phi]_{350}$ $+5000°$, $[\phi]_{308}$ $-6600°$, $[\phi]_{280}$ $+400°$.

Anal. Calcd. for $C_{16}H_{16}ClNO$: C, 70.20; H, 5.89. Found: C, 70.27; H, 6.13.

(*R*)-(−)-**N-5-Bromosalicylidene-α-phenylethylamine** (**VIa**).—Addition of (R)-(+)-α-phenylethylamine to a 13% excess of 5-bromosalicylaldehyde in methanol gave VIa (90% yield), yellow needles, m.p. 130–132° (95% ethanol), $[\alpha]^{25}D$ $-54°$, $[\phi]^{25}D$ $-164°$ (c 0.4, absolute ethanol); R.D. in 95% ethanol, 26°: (c 0.60) $[\phi]_{600}$ $-190°$, $[\phi]_{589}$ $-220°$, $[\phi]_{470}$ $-850°$; (c 0.096) $[\phi]_{470}$ $-1100°$, $[\phi]_{448}$ $-1400°$, $[\phi]_{409}$ $-760°$, $[\phi]_{370}$ $-2500°$; (c 0.0096) $[\phi]_{370}$ $-3200°$, $[\phi]_{351}$ $-6300°$, $[\phi]_{298}$ $+9500°$, $[\phi]_{275}$ $+1900°$.

Anal. Calcd. for $C_{15}H_{14}BrNO$: C, 59.22; H, 4.64. Found: C, 59.03; H, 4.93.

(±)-**N-5-Bromosalicylidene-α-phenylethylamine**.—Addition of a 56% excess of (±)-α-phenylethylamine to 5-bromosalicylaldehyde in 95% ethanol gave the Schiff base (91% yield), yellow needles, m.p. 105–106° (95% ethanol).

Anal. Calcd. for $C_{15}H_{14}BrNO$: C, 59.22; H, 4.64. Found: C, 58.97; H, 4.63.

(*S*)-(+)-**N-5-Bromosalicylidene-α-benzylethylamine** (**VIb**).—Addition of (*S*)-(+)-α-benzylethylamine to an 18% excess of 5-bromosalicylaldehyde in methanol gave VIb (86% yield), microscopic light yellow needles, m.p. 87–88° (95% ethanol), $[\alpha]^{25}D$ $+186°$, $[\phi]^{25}D$ $+592°$ (c 0.9, absolute ethanol); R.D. in 95% ethanol, 26°: (c 0.30) $[\phi]_{600}$ $+640°$, $[\phi]_{589}$ $+680°$, $[\phi]_{470}$ $+1900°$; (c 0.061) $[\phi]_{470}$ $+2200°$, $[\phi]_{444}$ $+2900°$, $[\phi]_{403}$ $+900°$, $[\phi]_{360}$ $+4400°$; (c 0.012) $[\phi]_{360}$ $+3700°$, $[\phi]_{349}$ $+5300°$, $[\phi]_{306}$ $-5000°$, $[\phi]_{280}$ $+2600°$.

Anal. Calcd. for $C_{16}H_{16}BrNO$: C, 60.39; H, 5.07. Found: C, 60.31; H, 5.24.

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Peptide Synthesis. II. Convenient Synthesis of *p*-Nitrobenzyl Esters of Amino Acids and Peptides

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p-Nitrobenzyl tosylate undergoes ready nucleophilic attack with displacement of the $(-OSO_2C_6H_4CH_3)^-$ ion when it is treated with the sodium or trialkylammonium salt of carbobenzoxyamino acids and peptides. The yields of *p*-nitrobenzyl esters thus prepared are consistently better than 70%. Included are the *p*-nitrobenzyl esters of carbobenzoxyglycine, carbobenzoxy-L-phenylalanine, and carbobenzoxy-L-threonine, and the dipeptide esters of carbobenzoxyglycyl-L-leucine and carbobenzoxy-L-prolyl-L-phenylalanine. By use of this procedure, N-tritylglycine and N-trityl-L-tryptophan were converted into the corresponding *p*-nitrobenzyl esters. Detritylation of the latter by mild acid solvolysis afforded the respective *p*-nitrobenzyl esters of glycine and L-tryptophan, both isolated as the *p*-toluenesulfonates. Similarly, the tripeptide derivative, N(im)-benzyl-L-histidyl-L-prolyl-L-phenylalanine *p*-nitrobenzyl ester di-*p*-toluenesulfonate, was prepared. Esterification of the C-terminal carboxyl end proceeded, in all cases tested, with no detectable amount of racemization.

During the course of synthetic studies with angiotensin analogs [2] carried out in this laboratory, it was found preferable to cover the C-terminal carboxyl group of amino acids and peptides by the *p*-nitrobenzyl group. In contrast to benzyl esters which are labile to anhydrous hydrogen bromide, the *p*-nitrobenzyl esters exhibit a marked stability to acid cleavage.[3] This permits a selective splitting of the N-carbobenzoxy group of an intermediate peptide, while retaining the C-terminal carboxyl end protected by the *p*-nitrobenzyl group. The latter is readily removed by catalytic hydrogenation.

Reports on the syntheses of *p*-nitrobenzyl esters involve the carbon tetrachloride azeotropic method[3] and the alkylation of N-acylamino acids with *p*-nitrobenzyl bromide or chloride in the presence of a tertiary base.[4]

The azeotropic method affords high yields, but when applied in the case of peptides, with prolonged heating for 2–3 days in acidic medium, it is not free of complications. Furthermore, this method is not suitable in the case of complex peptides having polyfunctional groups protected by various labile groups, like trityl or other acid sensitive groups. On the other hand, the direct alkylation with *p*-nitrobenzyl bromide or chloride would involve undesirable side reactions in the case of N-acylpeptides.

In view of the importance of preparing *p*-nitrobenzyl esters of peptides at any stage during synthetic work on polypeptides, the potentialities of *p*-nitrobenzyl tosylate as the alkylating agent have been investigated.

p-Nitrobenzyl tosylate was prepared some years ago by Tipson,[5] and more recently by Kochi and Hammond,[6] during kinetic studies of the solvolysis rates of tosylates. The method consisted of the tosylation of *p*-nitrobenzyl alcohol in dry pyridine. We did not pursue this method, however, since we have found that reaction of the silver *p*-toluenesulfonate with *p*-nitrobenzyl bromide affords the desired ester in high and reproducible yield. By Tipson's method the partial solvation of the so-formed *p*-nitrobenzyl tosylate by

(1) This investigation was supported in part by the Royal Hellenic Research Foundation, to which we are indebted.

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(4) (a) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **42**, 972 (1959); (b) H. Schwarz and K. Arakawa, *J. Am. Chem. Soc.*, **81**, 5691 (1959).

(5) R. S. Tipson, *J. Org. Chem.*, **9**, 235 (1944).

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pyridine is a possibility; hence the crude ester thus obtained requires several recrystallizations before analysis.[6]

The use of *p*-nitrobenzyl tosylate as the alkylating agent has given remarkable results. When the sodium or trialkylammonium salt of carbobenzoxy amino acids and peptides was heated for a short time with the equivalent amount of *p*-nitrobenzyl tosylate in acetone or acetone–dimethylformamide solution, the corresponding *p*-nitrobenzyl ester was obtained in high yield. As examples of N-acylamino acids, carbobenzoxyglycine, carbobenzoxy-L-phenylalanine, and carbobenzoxy-L-threonine were used; when these were treated by the procedure described in this paper, the corresponding *p*-nitrobenzyl esters were obtained in yields consistently better than 70%.

$$Z\text{—HNCH(R)COO}^{-}X^{+} + CH_3C_6H_4SO_2O^{-}\overset{+}{C}H_2C_6H_4NO_2$$

$$\downarrow \qquad\qquad \downarrow$$

$$Z\text{—HNCH(R)COOCH}_2C_6H_4NO_2 \qquad CH_3C_6H_4SO_2ONa$$

Z = carbobenzoxy; X = Na or $(C_2H_5)_3N$

In the case of carbobenzoxy-L-threonine, no side reaction with its secondary function was detected. The preparation of carbobenzoxy-L-threonine calls for some comment, since difficulty was experienced in obtaining pure crystalline material when carbobenzoxylation of L-threonine was conducted in sodium hydroxide or magnesium oxide solution.[7] However, when the reaction was carried out in dilute bicarbonate solution, the desired product, carbobenzoxy-L-threonine, was readily obtained in crystalline form.

In order to ascertain that no racemization takes place during the preparation of *p*-nitrobenzyl esters of N-acylamino acids, the crude esters of carbobenzoxy-L-phenylalanine and carbobenzoxy-L-threonine were hydrogenated over palladium black. The amino acids, which were recovered in 95% yield, exhibited the specific rotation of L-phenylalanine and L-threonine.

In addition to carbobenzoxy amino acids, N-trityl-amino acids have been esterified by the procedure reported in this paper. The sensitive N-trityl group survives during this esterification process and the corresponding N-tritylamino acid *p*-nitrobenzyl esters were readily obtained. With N-tritylglycine the yield of *p*-nitrobenzyl ester was 95%, while the yield from direct alkylation with *p*-nitrobenzyl bromide, after prolonged reaction time, was only 67%. Similarly, N-trityl-L-tryptophan was converted into its corresponding *p*-nitrobenzyl ester; subsequent facile removal of the N-trityl group by mild acid solvolysis afforded L-tryptophan *p*-nitrobenzyl ester *p*-toluenesulfonate in 50% over-all yield. Its absorption spectrum and chromatographic behavior on paper indicated neither oxindole nor any ninhydrin- nor Ehrlich-positive impurity.

Synthesis of L-tryptophan *p*-nitrobenzyl ester *p*-toluenesulfonate either by the azeotropic method[3] or by decarbobenzoxylation of the corresponding ester by hydrogen bromide should be met with reserve; the sensitivity of tryptophan derivatives to the action of hydrogen bromide is well known.[8]

(7) E. Baer and F. Eckstein, *J. Biol. Chem.*, **237**, 1449 (1962).
(8) D. Theodoropoulos and J. S. Fruton, *Biochemistry*, **1**, 933 (1962).

An important advantage in our procedure for the synthesis of *p*-nitrobenzyl esters lies in the fact that it is not restricted to the N-acylamino acid stage. This can be accomplished most satisfactorily with N-acylpeptides. Carbobenzoxyglycyl-L-leucine was readily converted into its corresponding *p*-nitrobenzyl ester in 87% yield. The product was an oil and exhibited an optical value of $[\alpha]^{27}D$ $-8.9° \pm 1°$ as 5.48% solution in ethyl acetate. Catalytic hydrogenation of it afforded glycyl-L-leucine, homogeneous according to paper chromatography and with specific rotation in agreement to that reported.[9]

Emphasis has been placed upon the synthesis of carbobenzoxy-L-prolyl-L-phenylalanine *p*-nitrobenzyl ester, since this peptide derivative contains the C-terminal amino acid sequence 7–8 of angiotensin.[10] Carbobenzoxy-L-prolyl-L-phenylalanine, reported[11] as an oil, was obtained in solid form by saponification of the corresponding methyl ester. The N-acyldipeptide, either *via* its sodium or trialkylammonium salt, reacted with *p*-nitrobenzyl tosylate to produce crystalline *p*-nitrobenzyl ester in 86% yield. The optical integrity of the ester was established by hydrogenating it to the free dipeptide, prolylphenylalanine. The latter was proved to be all L–L- in comparison with the optical value reported for this peptide.[11]

Extension of this esterification procedure to the synthesis of a tripeptide ester gave a quite remarkable yield considering the small amounts of reactants used (see Experimental). Thus, N-trityl-N(im)-benzyl-L-histidyl-L-prolyl-L-phenylalanine was converted to its corresponding *p*-nitrobenzyl ester, and the latter, by mild solvolysis in ethanol containing 2 equiv. of *p*-toluenesulfonic acid, afforded N(im)-benzyl-L-histidyl-L-prolyl-L-phenylalanine *p*-nitrobenzyl ester di-*p*-toluenesulfonate in 81% over-all yield. The above sequence is contained as amino acids 6–8 in isoleucine angiotensin[10] and this intermediate would serve in further studies with this hormone.

The above tripeptide acid, N-trityl-N(im)-benzyl-L-histidyl-L-prolyl-L-phenylalanine, was built up by condensation of L-prolyl-L-phenylalanine methyl ester hydrochloride[1] with N-trityl-N(im)-benzyl-L-histidine diethylammonium salt using dicyclohexylcarbodiimide[12] as condensing agent. The resulting ester, N-trityl-N(im)-benzyl-L-histidyl-L-prolyl-L-phenylalanine methyl ester, was then saponified with no difficulty to give the desired tripeptide acid.

In relation to the synthesis of histidyl peptides it should be emphasized that N-trityl-N(im)-benzyl-L-histidine diethylammonium salt may serve, in this connection, as an additional intermediate; other intermediates, like carbobenzoxy-N(im)-benzyl-L-histidine and N(im)-benzyl-L-histidine methyl and benzyl esters[13] have found considerable use in recent synthetic work.[14]

(9) H. Carpenter and T. Gish, *J. Am. Chem. Soc.*, **74**, 3818 (1952); D. Theodoropoulos and J. Gazopoulos, *J. Org. Chem.*, **27**, 2091 (1962).
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Experimental

p-Nitrobenzyl Tosylate.—A solution of silver nitrate (16.9 g., 0 1 mole) in 20 ml. of distilled water was mixed with sodium *p*-toluenesulfonate dihydrate (23 g., 0.1 mole) and dissolved in an equal amount of water with stirring. The precipitate thus formed was filtered by suction, washed with 5 ml. of cold water, and dried *in vacuo* over P_2O_5 in the absence of light yielding 21 g. (75%), m.p. 225° dec. The silver salt (15.3 g.) was suspended in 100 ml. of dry chloroform containing 11 g. of *p*-nitrobenzyl bromide and the mixture was boiled under reflux for 24 hr. in the dark. The reaction mixture was then filtered, the filtrate was evaporated *in vacuo*, and the remaining residue was filtered by addition of cold petroleum ether (b.p. 50–70°); 13.6-g. (88%) yield, m.p. 86–88°. One recrystallization from isopropyl alcohol–petroleum ether yielded 12.1 g. (80%), m.p. 103° (lit.[6] m.p. 103–105°.) The crude ester, m.p. 86–88°, was pure enough and used for further processing.[15]

Carbobenzoxy-L-threonine. In a 1-l. round flask equipped with a mechanical stirrer and immersed in an ice–water bath, were placed 13 g. (0.1 mole) of L-threonine, $[\alpha]^{27}D$ −26.9 ± 0.5° (*c* 3, water), and 400 ml. of 0.4 *M* sodium bicarbonate solution. To the well-stirred mixture was added 20 ml. (20% excess) of carbobenzoxy chloride over a period of 2 hr. (1 ml. every 6 min.). After this time the stirring was continued for an additional hour. The reaction mixture was then extracted twice with ether and the water layer was acidified to congo red with hydrochloric acid. The oily precipitate was cooled in the refrigerator overnight, and the next day upon scratching with a glass rod began to crystallize. The crude product weighed 12 g. and had m.p. 102–103° (lit.[7] m.p. 103–104°). Extraction of the water layer with ethyl acetate, drying of the solvent, and evaporation to dryness afforded an additional amount of 7 g. of product, m.p. 99–101°.

Carbobenzoxy-L-threonine p-Nitrobenzyl Ester.—To a solution of 2.7 g. (0.01 mole) of carbobenzoxy-L-threonine in 5 ml. of absolute methanol was added 0.55 g. (0.01 mole) of sodium methylate and the mixture evaporated to dryness. The residue was almost dissolved in 15 ml. of acetone–dimethylformamide (2:1), 3.07 g. (0.01 mole) of *p*-nitrobenzyl tosylate (crude ester) was added, and the mixture was boiled under reflux. After 5 min. a fluffy insoluble material (sodium *p*-toluenesulfonate) began to appear. Boiling was continued for 30 min. and then insoluble material was filtered off and the filtrate was evaporated *in vacuo* at temperature never above 40°. When acetone was removed, the remaining dimethylformamide solution was diluted with 500 ml. of water, The product crystallized immediately; for complete precipitation, however, it was cooled for several hours in the refrigerator. It was filtered, washed well with bicarbonate solution and water, and dried, yielding 3 g. (77%), m.p. 95–97°. On recrystallization from ethanol–ether–petroleum ether (1:1:2) it yielded 2.4 g. (61%), m.p. 111–112°, $[\alpha]^{25}D$ −12.4 ±0.5° (*c* 2.5, chloroform). Its absorption spectrum in ethanol indicated a minimum at 230 mμ and a maximum at 270 mμ (ϵ_{mol} 9250).

Anal. Calcd. for $C_{19}H_{20}N_2O_7$: C, 58.7; H, 5.1; N, 7.2. Found: C, 58.8; H, 5.3; N, 7.1.

A solution of 1 g. of carbobenzoxy-L-threonine *p*-nitrobenzyl ester (crude product) in acetic acid–water was hydrogenated over palladium black. The catalyst was removed by filtration and washed several times with acetic acid–water, and the combined filtrate was evaporated to dryness. The peptide, which was isolated by addition of acetone, weighed 0.287 g. (95%) and without recrystallization possessed specific rotation $[\alpha]^{25}D$ −26.2 ± 0.5° (*c* 3, water).

Carbobenzoxy-L-phenylalanine p-Nitrobenzyl Ester.—Carbobenzoxy-L-phenylalanine (2.96 g., 0.01 mole) and *p*-nitrobenzyl tosylate (3.07 g., 0.01 mole) were treated according to the procedure described above, yielding 3.5 g. (81%), m.p. 97–99°. The product was recrystallized from benzene–petroleum ether, yielding 3.2 g. (75%), m.p. 105–106°, $[\alpha]^{27}D$ +7.1 ±0.5° (*c* 3, chloroform).

Anal. Calcd. for $C_{24}H_{22}N_2O_6$: C, 66.3; H, 5.1; N, 6.4. Found: C, 66.4; H, 5.1; N, 6.6.

Hydrogenolysis of 1 g. of carbobenzoxy-L-phenylalanine *p*-nitrobenzyl ester (crude product), in the usual manner, afforded 0.341 g. (93%) of L-phenylalanine with specific rotation of $[\alpha]^{26}D$ −35.3 ± 0.5° (*c* 1.6, water). Authentic L-phenylalanine, used as the starting material, exhibited $[\alpha]^{26}D$ −35.5 ± 0.5° (*c* 1.6, in water).

Carbobenzoxyglycine p-Nitrobenzyl Ester. A.—This compound was prepared in a manner similar to that outlined above, yielding 2.7 g. (80%), m.p. 107–109° (lit.[4b] m.p. 107–109.5°).

B.—Carbobenzoxyglycine (2 g., 0.01 mole) was dissolved in dry acetone by addition of 1.01 g. (0.01 mole) of triethylamine. To this solution 3.07 g. (0.01 mole) of *p*-nitrobenzyl tosylate was added and the mixture was boiled under reflux for 1 hr. During that time triethylammonium *p*-toluenesulfonate precipitated. The reaction mixture was filtered, the filtrate was evaporated to dryness, and the residue, upon addition of water and cooling, crystallized. It was washed with dilute bicarbonate solution and water, and dried, yielding 3.1 g. (90%). It was recrystallized from isopropyl alcohol, yielding 2.6 g. (78%), m.p. 107–109°.

Anal. Calcd. for $C_{17}H_{16}N_2O_6$: C, 59.2; H, 4.6; N, 8.1. Found: C, 59.3; H, 4.6; N, 8.3.

N-Tritylglycine p-Nitrobenzyl Ester. A.—Treatment of the sodium or triethylammonium salt of N-tritylglycine in similar fashion afforded the corresponding *p*-nitrobenzyl ester in 95% yield. The crude product was dissolved in ethyl acetate and this solution was washed with 5% aqueous diethylamine solution and water, and dried. Removal of the solvent *in vacuo* gave 4.2 g. (93%) of product, m.p. 106–107°. It was recrystallized from chloroform–ether–petroleum ether (1:2:3), in 80% yield, m.p. 125–126°.

B.—A solution of N-tritylglycine (3.17 g., 0.01 mole), triethylamine (1.01 g., 0.01 mole) and *p*-nitrobenzyl bromide (2.1 g., 0.01 mole) in 10 ml. of dry chloroform was boiled under reflux for 6 hr. and then kept at room temperature for an additional 24 hr. The solution was diluted with 100 ml. of chloroform and this solution was washed with 5% aqueous diethylamine and water, and dried. The solvent was evaporated *in vacuo* and upon addition of petroleum ether the residue crystallized, yielding 2.9 g. (64%), m.p. 126°.

Anal. Calcd. for $C_{28}H_{24}N_2O_4$: C, 74.3; H, 5.4; N, 6.1. Found: C, 74.2; H, 5.2; N, 6.2.

Glycine p-Nitrobenzyl Ester p-Toluenesulfonate.—A solution of N-tritylglycine *p*-nitrobenzyl ester (2.2 g., 0.005 mole) and 0.95 g. (5% excess) of *p*-toluenesulfonic acid monohydrate in 10 ml. of acetone was boiled under reflux for 10 min. During this time a white precipitate was formed which was filtered and washed with dry ether, yielding 1.95 g. (96%), m.p. 200–201°. A sample recrystallized from isopropyl alcohol–ether had m.p. 200–202.

Anal. Calcd. for $C_{16}H_{18}N_2O_7S$: C, 51.6; H, 4.8; N, 7.5. Found: C, 51.6; H, 4.8; N, 7.1.

L-Tryptophan p-Nitrobenzyl Ester p-Toluenesulfonate.—Using 2.2 g. (0.005 mole) of N-trityl-L-tryptophan diethylammonium salt,[16] N-trityl-L-tryptophan *p*-nitrobenzyl ester (2.5 g.) was obtained in the same manner described for the threonine derivative. The crude ester (2.5 g.) was dissolved in 5 ml. of acetone, a few drops of diethylamine and 5 ml. of dry ether were added, and the solution was cooled in the refrigerator overnight. The resulting slight precipitate was removed by filtration, the filtrate was evaporated to dryness, and the residue was solidified by addition of water, yielding 2.4 g. (dried over P_2O_5 *in vacuo*). Detritylation was effected by dissolving it in 10 ml. of ethanol, adding 0.9 g. of *p*-toluenesulfonic acid monohydrate, and boiling for 5 min. Ethanol was removed *in vacuo* and replaced by dry ether. The resulting oil was triturated with a glass rod and the solvent was decanted. Addition of isopropyl alcohol caused the oily product to crystallize, yielding 1.2 g. (50%), m.p. 200–202°. On recrystallization from ethanol–ether it had m.p. 206–207°, $[\alpha]^{25}D$+12.8 ± 0.5° (*c* 2, dimethylformamide). An 80% ethanolic solution of the product gave an absorption spectrum with a maximum at 280 mμ (ϵ_{mol} 13,800) and a minimum at 235 mμ (ϵ_{mol} 1950). Paper chromatography in 1-butanol–pyridine–acetic acid–water (15:10:3:12) revealed only one ninhydrin- and Ehrlich-positive spot, R_f 0.94. In the latter chromatographic system L-tryptophan had R_f 0.51.

Anal. Calcd. for $C_{25}H_{25}N_3O_7S$: C, 58.6; H, 4.9; N, 8.2. Found: C, 58.4; H, 5.2; N, 8.5.

(15) Attempted synthesis of benzyl tosylate from silver *p*-toluenesulfonate and benzyl chloride or bromide, under exactly the same conditions, resulted in the formation of a pitch-black product. It seems that prolonged heating of the reaction mixture favors self-alkylation of the so-formed benzyl tosylate *via* formation of benzyl carbonium ions $ArCH_2^+$; this is in line with the varying stability of tosylates.[6]

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Carbobenzoxyglycyl-L-leucine *p*-Nitrobenzyl Ester.—Starting with 1.8 g. (0.005 mole) of carbobenzoxyglycyl-L-leucine, m.p. 101–102°, $[\alpha]^{26}D$ −18.5 ± 0.5° (*c* 1.39, *N* NaOH), the *p*-nitrobenzyl ester was obtained as a waxy product, yield 2.2 g. (96%), $[\alpha]^{27}D$ −8.9 ± 1° (*c* 5.48, ethyl acetate).

Hydrogenolysis of the above ester (2.2 g.) in the usual manner afforded 1.2 g. (70%) of glycyl-L-leucine (crude product), $[\alpha]^{25}D$ −36° (*c* 2, water).

Carbobenzoxy-L-prolyl-L-phenylalanine.—Carbobenzoxy-L-prolyl-L-phenylalanine methyl ester (2.05 g., 0.005 mole), obtained by condensation of carbobenzoxy-L-proline with L-phenylalanine methyl ester by the dicyclohexylcarbodiimide method,[12] was dissolved in 10 ml. of ethanol and 5 ml. of *N* sodium hydroxide was added. After 1 hr. the solution was diluted with 50 ml. of water and unhydrolyzed material was extracted with ether. The water layer was acidified to congo red with hydrochloric acid and the resulting oil was taken up in ether. The ethereal solution was extracted with dilute bicarbonate solution; the latter was acidified with hydrochloric acid. The oily product was taken up in ether and this solution was washed with water until it was free from acid and then was dried with anhydrous sodium sulfate. The solvent was evaporated to dryness and the residue was kept *in vacuo* over P_2O_5 for 24 hr. The substance then solidified and had m.p. 123–124°, yield 1.4 g. (73%), $[\alpha]^{20}D$ −36.6 ± 0.5° (*c* 2, chloroform).

Anal. Calcd. for $C_{22}H_{24}N_2O_5$: C, 66.6; H, 6.1; N, 7.0. Found: C, 66.3; H, 6.2; N, 6.8.

Carbobenzoxy-L-prolyl-L-phenylalanine *p*-Nitrobenzyl Ester.—A solution of 1.98 g. (0.005 mole) of carbobenzoxy-L-prolyl-L-phenylalanine, 0.5 g. (0.005 mole) of triethylamine and 1.5 g. (0.005 mole) of *p*-nitrobenzyl tosylate in 10 ml. of dry acetone was boiled under reflux for 1 hr. The solvent was evaporated to dryness and replaced with ethyl acetate. This solution was washed well with dilute bicarbonate solution and water, and dried. The solvent was removed *in vacuo* and the remaining residue, upon addition of a few milliliters of isopropyl alcohol and cooling, was crystallized, yielding 2.3 g. (86%), m.p. 86–87°. On recrystallization from isopropyl alcohol–petroleum ether it had m.p. 87–89°.

Anal. Calcd. for $C_{29}H_{29}N_3O_7$: C, 65.5; H, 5.4; N, 7.9. Found: C, 65.7; H, 5.5; N, 8.0.

A portion of the above ester (1 g.) was hydrogenated over palladium black in ethanol–acetic acid solution. The dipeptide (0.3 g.), L-prolyl-L-phenylalanine, thus obtained had $[\alpha]^{20}D$ −39.6 ± 1° (*c* 1.7, 6 *N* HCl). This optical value was in good agreement to that reported for the recrystallized product.[11]

N-Trityl-N(im)benzyl-L-histidine Diethylammonium Salt.—To a solution of N(im)benzyl-L-histidine[17] (2.54 g., 0.01 mole), diethylamine (3 ml., 0.03 mole), water (4 ml.), and isopropyl alcohol (8 ml.) was added trityl chloride (3.6 g., 0.013 mole) with shaking over a period of 1 hr. at room temperature. Water (10 ml.) was then added and the reaction mixture was extracted with two 50-ml. portions of chloroform. The chloroform extract was dried over sodium sulfate and the solvent was evaporated to dryness. The remaining residue, upon addition of dry ether and cooling overnight, was crystallized, yielding 1.4 g. (25%), m.p. 135–137°. On recrystallization from isopropyl alcohol–ether it had m.p. 146–148° (dried under high vacuum over P_2O_5), yield 1.3 g., $[\alpha]^{20}D$ +14.7 ± 0.5° (*c* 1.5, chloroform).

Anal. Calcd. for $C_{36}H_{40}N_4O_2$: C, 77.1; H, 7.1; N, 9.9. Found: C, 77.4; H, 7.2; N, 10.1.

N-Trityl-N(im)-benzyl-L-histidyl-L-prolyl-L-phenylalanine.—N-Trityl-N(im)-benzyl-L-histidine diethylammonium salt (2.8 g., 0.005 mole) and L-prolyl-L-phenylalanine methyl ester hydrochloride[2] (1.56 g., 0.005 mole) were coupled by means of dicyclohexylcarbodiimide (1 g.) in methylene chloride. After 24 hr. the filtrate was washed with two 25-ml. portions of 5% aqueous diethylamine solution and water, dried, and then concentrated *in vacuo* at 35°. The remaining sirupy ester (2.9 g.) was saponified with 4.2 ml. (10% excess) of 1 *N* NaOH in 5 ml. of ethanol. After 1 hr. the reaction mixture was diluted with 30 ml. of water and acidified with acetic acid. The resulting precipitate was filtered, washed well with water, and dried, yielding 2.4 g. For purification it was dissolved in ethyl acetate (5 ml.), diethylamine (0.5 ml.) was added, and the solution was kept in the refrigerator overnight. The diethylammonium salt of N-trityl-N(im)-benzyl-L-histidyl-L-prolyl-L-phenylalanine, which was precipitated completely by addition of 10 ml. of ether, was isolated by filtration; it was then suspended in water and acidified with acetic acid. The product thus obtained weighed 2.3 g. (82%), $[\alpha]^{22}D$ −5.5° (*c* 4, chloroform), and melted completely at 145°, with previous softening at 130°.

Anal. Calcd. for $C_{46}H_{45}N_5O_4$: C, 75.4; H, 6.1; N, 9.5. Found: C, 75.1; H, 7.0; N, 9.7.

N(im)-Benzyl-L-histidyl-L-prolyl-L-phenylalanine *p*-Nitrobenzyl Ester Di-*p*-Toluenesulfonate.—To a solution of 0.9 g. of N-trityl-N(im)-benzyl-L-histidyl-L-prolyl-L-phenylalanine and 0.17 ml. of triethylamine in 5 ml. of dry acetone was added 0.38 g. of *p*-nitrobenzyl tosylate. The mixture was boiled under reflux over a period of 1 hr. and then the solvent was evaporated *in vacuo*. The remaining residue was taken up in ethyl acetate and this solution was washed with 5% aqueous diethylamine and water, and dried. After evaporation of the solvent the residue was dissolved in 5 ml. of ethanol and detritylated by addition of 0.48 g. of *p*-toluenesulfonic acid monohydrate and boiling of the reaction mixture for 5 min. under reflux. Ethanol was then removed *in vacuo* and a few milliliters of isopropyl alcohol were added. Upon scratching with a glass rod the product began to crystallize. Complete crystallization, however, was effected by addition of dry ether and cooling; the over-all yield was 81% (0.97 g.), m.p. 170–171° (softens at 110°). On recrystallization from isopropyl alcohol–ether the product (0.87 g.) had identical m.p. 170–171°, $[\alpha]^{20}D$ −8.7 ± 1° (*c* 1, dimethylformamide), −20.1 ± 0.5° (*c* 3, 50% acetic acid). Paper chromatography (Whatman No. 1) in 1-butanol–pyridine–acetic acid–water (15:10:3:12) revealed only one ninhydrin-positive spot, R_f 0.97. The data from elementary analysis accorded best with that calculated for the monohydrate.

Anal. Calcd. for $C_{48}H_{52}N_6O_{12}S_2 \cdot H_2O$: C, 58.4; H, 5.5; N, 8.5. Found: C, 58.2; H, 5.2; N, 8.2.

(17) V. du Vigneaud and O. Behrens, *J. Biol. Chem.*, **117**, 27 (1937).