palladium catalyst in the usual manner leads to the ultimate procurement of L-arginyl-L-glutamic acid[18] in nearly quantitative yield.

IV. Properties of Nα,Nω-Dicarbobenzoxyanhydro-L-arginine. Behavior toward Water.—A solution of Nα,Nω-dicarbobenzoxyanhydro-L-arginine in acetone is treated with an amount of water just sufficient to keep the material in solution. After storage at room temperature for 24 hours, the acetone is removed by evaporation under reduced pressure and the precipitate recovered by filtration. Analysis of the precipitate identified it as the unchanged starting material in nearly quantitative yield; $[\alpha]^{25}D$ −13.0° (4% in chloroform).

Action of Alkali.—A solution of Nα,Nω-dicarbobenzoxyanhydro-L-arginine in acetone is treated with 1 equivalent of aqueous alkali and stored at room temperature for 1 hour. After removal of the acetone under reduced pressure at a temperature not exceeding 25°, the residual material is acidified with acetic acid. The sirupy deposit is washed with a small volume of water and then dissolved in hot methanol. Upon cooling, crystalline Nα,Nω-dicarbobenzoxy-L-arginine precipitates in some 85–90% yield, m.p. 150°, $[\alpha]^{25}D$ −5.2° (2% in dimethylformamide) and $[\alpha]^{25}D$ −10.9° (1% in pyridine).

Degradation with Acetic Anhydride.—To 2.6 g. of Nα,Nω-dicarbobenzoxyanhydro-L-arginine is added 50 ml. of warm acetic anhydride. The reaction mixture is heated at 100° for 3 hours[31] and then cooled to room temperature. After the addition of a small volume of ethanol and water, the solution is permitted to stand at room temperature with occasional cooling; by such means most of the acetic anhydride is destroyed. The solution is then evaporated to dryness, the residue is dissolved in about 40 ml. of acetone and the latter solution is treated with 25 ml. of water and permitted to stand at room temperature in an open vessel. After a few hours, precipitation commences due to a slow evaporation of the acetone. Two days later, the crystalline deposit is collected by filtration and recrystallized from hot ethanol; yield 1 g., m.p. 141°. Elemental analysis of the product agree with the values calculated for N-acetyl-N'-carbobenzoxyurea.

Anal. Calcd. for $C_{11}H_{12}N_2O_4$: C, 55.9; H, 5.1; N, 11.9. Found: C, 56.3; H, 5.2; N, 11.9.

The mother liquors from the above reaction are kept at room temperature for one week, during which time a small amount of crystals precipitate. These are filtered off and the filtrate is concentrated to dryness under reduced pressure. A residue remains which is dissolved in approximately 20 ml. of 5 *N* HCl and then stored at room temperature for several days. During this time crystalline needles precipitate which, after storage in the cold, are collected by filtration and washed with a small amount of cold water; yield 0.5 g., m.p. 187–189°, $[\alpha]^{25}D$ −11.5° (1.0% in water). Elemental analysis of the product identified it as Nα-carbobenzoxy-ornithine hydrochloride.

Anal. Calcd. for $C_{13}H_{18}N_2O_4$.HCl: C, 51.6; H, 6.3; N, 9.2; Cl, 11.7. Found: C, 51.7; H, 6.4; N, 9.2; Cl, 11.9.

(31) Optical rotation measurements of the reaction mixture indicate no difference in reading at the end of 15 minutes, 2 hours and 3 hours of heating.

BETHESDA 14, MD.

CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY, UNIVERSITY OF ATHENS, GREECE]

On the Trityl Method for Peptide Synthesis[1]

BY GERASSIMOS C. STELAKATOS,[2] DIMITRIOS M. THEODOROPOULOS[3] AND LEONIDAS ZERVAS[4]

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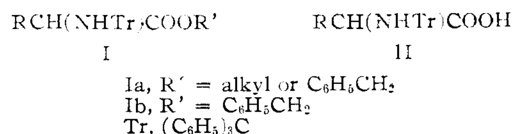
Trityl derivatives of amino acids can be simply and effectively isolated by chloroform extraction of their diethylammonium salts from the tritylation reaction mixture. N,N'-Ditrityl-L-histidine, N-trityl-L-histidine, N'-trityl-L-histidine methyl ester hydrochloride, O-trityl-L-tyrosine benzyl ester hydrochloride, amongst other tritylamino acids, were prepared. Coupling of tritylamino acids and trityldipeptides with amino acid esters was achieved *via* the carbodiimide method and the phosphorazo method. Trityl dipeptides may alternatively be coupled *via* the mixed anhydride procedure without any influence of the bulky trityl group.

Introduction

As was reported in an earlier communication,[5] the applicability of the trityl function in synthetic peptide techniques was limited not only by the difficulty generally encountered in the preparation of optically active tritylated amino acids (II) but by the failure of these compounds (with the exception of tritylalanine and tritylglycine), as well, to couple with other amino acids *via* the mixed anhydride procedure. The tritylation of amino acid esters proceeds with facility and in satisfactory yield; however, the appreciable steric hindrance afforded by the bulky trityl group makes the saponification of tritylamino acid esters (Ia) under mild conditions difficult and directs the coupling of mixed anhydrides toward carbamate rather than peptide formation.[5] Pure, optically active tritylamino acids (II) may be prepared by tritylation of the benzyl esters of amino acids (Ib) followed by catalytic hydrogenolysis of the



tritylamino acid benzyl ester so derived.[1,2,6] By virtue of the markedly greater rate of hydrogenolysis exhibited by the O-benzyl than the N-trityl substituent, cessation of the hydrogenation after the uptake of approximately 1 mole of hydrogen permits the ultimate isolation of the desired tritylamino acid (II) in satisfactory yield. Prolonged hydrogenolysis, on the other hand, leads to the re-

(1) Previous presentation of this work was given at the XIV International Congress of Pure and Applied Chemistry, Zurich, July 27, 1955; *cf.* Communications du XIV Congrès International de Chimie Pure et Appliqué, Zurich, 1955, p. 224.

(2) This paper is based in part on the doctoral dissertation of G. C. Stelakatos, Division of Natural Sciences (Chemistry Section), University of Athens, Greece, October, 1954. Now at Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda Md.

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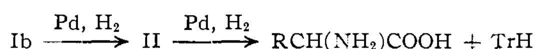
(4) This investigation was supported by a grant from the Rockefeller Foundation, to which I am greatly indebted.

(5) L. Zervas and D. M. Theodoropoulos, THIS JOURNAL, **78**, 1359 (1956).

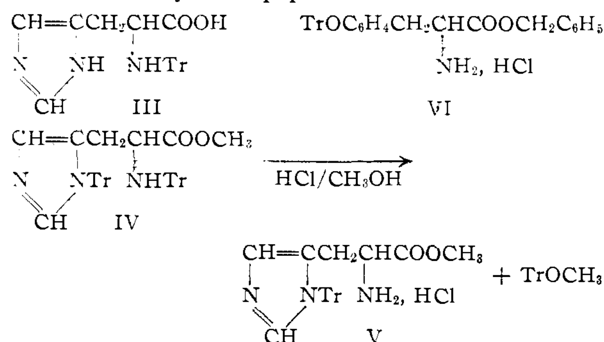
(6) G. Amiard, R. Heymes and L. Velluz, *Bull. soc. chim. France*, 698 (1956).

moval of the trityl group in the form of triphenylmethane.[5]



Preparation of tritylamino acids may be alternatively effected through direct tritylation of an amino acid in aqueous solution in the presence of diethylamine, followed by acidification of the reaction mixture with acetic acid.[1,5,6] However, the isolation of tritylamino acids may be most simply and effectively achieved in the form of their diethylammonium salts by extraction of the reaction mixture with chloroform. This latter modification, indeed, has extended the applicability of the method and has permitted the synthesis of the trityl derivatives of L-valine, L-alanine, L-leucine, L-tryptophan, L-methionine, L-glutamine, L-proline, L-isoleucine and L-histidine in addition to those previously reported.[5]

During the direct tritylation of L-histidine a small amount of monotrityl-L-histidine was isolated in addition to ditrityl derivative. The monotrityl derivative exhibits a positive Pauly test thus demonstrated the structure as III. On the other hand, when an absolute alcoholic solution of the hydrochloride ditrityl-L-histidine methyl ester (IV) was heated briefly on a steam-bath, detritylation occurred only at the α-amino position[3] with the formation of the monotrityl derivative V, which did not give a Pauly reaction. In a similar manner, O-trityl-L-tyrosine benzyl ester (VI) was prepared by mono-detritylation of the corresponding O,N ditrityl derivative. As these compounds (V and VI) contain a protected imidazole and a protected hydroxy function, respectively, they are of potential utility as intermediates in the synthesis of histidine and tyrosine peptides.



The difficulty encountered in the coupling of a tritylamino acid (II) with a large R group by the mixed anhydride procedure does not necessarily apply to other methods of coupling,[1,2,6] *e.g.* the phosphorazo method[7] or the carbodiimide method,[8] the mechanisms of which presumably are not as greatly influenced by the steric effect of the trityl group. The trityl derivatives of L-asparagine, L-glutamine and L-phenylalanine may thereby be condensed with other amino acids, although the yields are low. As was expected, no comparable steric effect could be detected when trityldi- or polypeptides were used *in lieu* of the amino acid derivatives. For this reason, coupling reactions involving the former derivatives proceed normally and in good yield, even in the case of the mixed anhydride method. The practical value of the trityl method therefore appears to lie chiefly in the synthesis of higher peptides[5] where the steric effect of the bulky trityl substituent is obviated, although its use for the synthesis of smaller peptides in special cases should not be precluded.

(7) S. Goldschmidt and H. Lautenschlager, *Ann.*, **580**, 68 (1953).

(8) J. C. Sheehan and G. P. Hess, THIS JOURNAL, **77**, 1067 (1955).

Experimental

Prior to analysis the trityl derivatives were dried at room temperature under a high vacuum; other compounds were dried at 78°.

Benzyl Esters of N-Tritylamino Acids (Ib).—The benzyl esters of the amino acid hydrochlorides, benzenesulfonates or *p*-toluenesulfonates may be used in the preparation of these compounds; their tritylation was carried out in exactly the same manner as the tritylation of the corresponding alkyl esters.[5]

N-Tritylglycine benzyl ester, yield 80%, m.p. 70°. *Anal.* Calcd. for $C_{28}H_{25}O_2N$: C, 82.5; H, 6.2; N, 3.4. Found: C, 82.3; H, 6.1; N, 3.4.

N-Trityl-L-leucine benzyl ester, yield 80%, m.p. 100–101°. *Anal.* Calcd. for $C_{32}H_{33}O_2N$: C, 82.9; H, 7.2; N, 3.0. Found: C, 82.6; H, 7.0; N, 2.8.

N-Trityl-L-phenylalanine benzyl ester, yield 75%, sirupy. *Anal.* Calcd. for $C_{35}H_{31}O_2N$: C, 85.5; H, 6.3; N, 2.8. Found: C, 85.2; H, 6.1; N, 2.9.

N-Trityl derivatives of glycine, L-alanine, L-phenylalanine, L-leucine, L-valine, L-methionine, L-asparagine, L-glutamine, L-proline, L-isoleucine and L-tryptophan were prepared by catalytic hydrogenation of the corresponding benzyl esters or by tritylation of the corresponding amino acids. The following procedures (A and B) are typical; the tritylamino acids thus prepared were isolated in the form of diethylammonium salts and are listed in Table I.

A. N-Trityl-L-phenylalanine benzyl ester (5 g., 0.01 mole) dissolved in methanol or ethyl acetate was hydrogenated in the presence of 0.5 g. of freshly prepared palladium black. After 30–45 min., 275 ml. (approx. 1.1 moles) of hydrogen had been absorbed (24°, 756 mm.); the hydrogenation was interrupted at this point. The solution was evaporated to dryness. Upon dissolving the residue in anhydrous ether, filtering off traces of phenylalanine and adding diethylamine, the diethylammonium salt of trityl-L-phenylalanine precipitated.

B. L-Leucine (1.3 g., 0.01 mole) was dissolved in a mixture of 4 ml. of water, 3 ml. (0.03 mole) of diethylamine and 8 ml. of isopropyl alcohol. Trityl chloride (3.6 g., 0.013 mole) was then added with continuous, vigorous shaking. The addition was effected in twelve equal portions within a period of one hour at room temperature. When the reaction was completed, 30 ml. of water was added and the mixture extracted twice with chloroform. The combined chloroform extracts, which contain tritylcarbinol and tritylmethylamine,[5] besides the desired product, were washed with a little water, dried over sodium sulfate and then evaporated to dryness *in vacuo*. Complete removal of chloroform was ensured by the addition of a few ml. of alcohol and repetition of the evaporation *in vacuo*. Upon dissolving the residue in anhydrous ether, adding a few drops of diethylamine, and cooling the solution, the diethylammonium salt of trityl-L-leucine precipitated; the latter was washed repeatedly with anhydrous ether, wherein the above-mentioned by-products are soluble. The water layer was acidified with acetic acid and evaporated to dryness *in vacuo;* after the addition of alcohol, 0.7 g. of L-leucine was recovered.

N-Trityl-L-asparagine.—L-Asparagine hydrate (1.5 g., 0.01 mole) was tritylated by procedure B, as described above. At the end of the tritylation water was added. The precipitate, consisting mostly of triphenylcarbinol, was filtered off and washed with water containing a few drops of diethylamine. The combined filtrates were acidified with 10 ml. of acetic acid in the cold and immediately extracted twice with chloroform (30 ml. each time). The combined chloroform extracts were washed twice with water and dried for a few minutes over sodium sulfate. On standing in the cold, trityl-L-asparagine crystallized out; yield 1.8 g. (48%), m.p. 175°, $[\alpha]^{25}D$ −6.3° (*c* 4%, in methanol); reported[5] m.p. 174°, $[\alpha]^{20}D$ −6.1° (in methanol).

TABLE I

Diethylammonium salts of N-tritylamino acids[a]	Yield,[b,c,d] %	M.p., °C.	$[\alpha]_D$[e]	Formula	Carbon, % Calcd.	Found	Hydrogen, % Calcd.	Found	Nitrogen, % Calcd.	Found
Glycine	80[b]	132[f]		$C_{25}H_{30}O_2N_2$					7.2	7.3
L-Alanine	50,[c] 80[d]	157[g]	−18.7[g]	$C_{26}H_{32}O_2N_2$					6.9	6.8
L-Valine	25,[c] 70[d]	160	+ 6.7	$C_{28}H_{36}O_2N_2$	77.7	77.5	8.4	8.2	6.5	6.3
L-Leucine	75,[b] 28,[c] 90[d]	154[h]	+ 3.0[h]	$C_{29}H_{38}O_2N_2$					6.3	6.5
L-Isoleucine	20,[c] 60[d]	150–152	+13.0	$C_{29}H_{38}O_2N_2$	78.0	77.8	8.6	8.5	6.3	6.2
L-Methionine	20,[c] 50[d]	152–153	+21.7	$C_{28}H_{36}O_2N_2S$	72.4	72.3	7.8	7.9	6.0	5.9
L-Phenylalanine	70,[b] 40,[c] 75[d]	150[i]	+12.5[i]	$C_{32}H_{36}O_2N_2$					5.8	5.7
L-Tryptophan	47,[c] 70[d]	150–151	+ 4.5	$C_{34}H_{37}O_2N_3$	78.6	78.8	7.2	7.3	8.1	8.0
L-Asparagine	40,[c] 70[d]	150[j]		$C_{27}H_{33}O_3N_3$					9.4	9.2
L-Glutamine	35[c]	142–144	+21.9	$C_{28}H_{35}O_3N_3$	72.8	72.9	7.6	7.8	9.1	9.0
L-Proline	50[c]	163–165	−57.5	$C_{28}H_{34}O_2N_2$	78.2	78.1	8.0	7.9	6.5	6.3

[a] Recrystallized from acetone. [b] Yield by method A. [c] Yield by method B. [d] Yield by method B calculated on the basis of amino acid reacted, as determined by the amount of amino acid recovered. [e] *c* 5%, in methanol; for the proline derivative chloroform was used. [f] Reported[5] m.p. 132°. [g] Reported[5] m.p. 157°, $[\alpha]_D$ −18.9°, in methanol. [h] Reported[5] m.p. 154–155°, $[\alpha]_D$ +2.8°, in methanol. [i] Reported[5] m.p. 150–151°, $[\alpha]_D$ +12.2°, in methanol. [j] Reported[5] m.p. 150–151°.

N-Trityl-L-phenylalanine.—A. To a suspension of 4.8 g. (0.01 mole) of trityl-L-phenylalanine diethylammonium salt in 100 ml. of water was added 10 ml. of 1 *N* hydrochloric acid under cooling and shaking. The mixture was immediately extracted with ether and the ether layer was washed with water, dried over sodium sulfate and evaporated to dryness. Upon prolonged drying in the high vacuum at room temperature the substance crystallized in the form of prisms; the yield was 3.8 g. (95%), m.p. 187–188° (after softening at about 100°), $[\alpha]^{26}_D$ +32.8° (*c* 10%, in chloroform); reported[5] m.p. 185–188°.

B. The above diethylammonium salt (4.8 g.) was dissolved in 250 ml. of 0.05 *N* sodium hydroxide and the solution was kept in vacuum at room temperature until the diethylamine was removed. Upon acidification with acetic acid, trityl-L-phenylalanine precipitated in almost quantitative yield; it was dried first on a porcelain plate and then in high vacuum over phosphorus pentoxide. Even under these conditions, the substance still contained some water which could be removed only by dissolving the substance in benzene or ether and repeating the evaporation *in vacuo;* m.p. 186–188°.

N-Trityl-L-histidine (III) and N,N'-Ditrityl-L-histidine.—L-Histidine monohydrochloride hydrate (2.1 g., 0.01 mole) was dissolved in a mixture of 10 ml. of aqueous *N* sodium hydroxide, 6 ml. (0.06 mole) of diethylamine and 16 ml. of isopropyl alcohol and the solution tritylated with 7.2 g. (0.026 mole) of trityl chloride as described. At the end of the reaction, water was added. The precipitate was filtered off, washed with water, dried and then dissolved in hot acetone. On standing at room temperature, ditrityl-L-histidine diethylammonium salt mixed with free acid precipitated out. This was filtered off, washed with acetone, and dissolved in alcoholic potassium hydroxide. The solution was kept *in vacuo* at 25° until the diethylamine was removed. Upon adding water and acidifying with acetic acid, 2.2 g. (35%) of the ditrityl-L-histidine crystallized out; this was washed successively with water, alcohol and ether; prisms, m.p. 198–200° (reported[9] 184–185°), $[\alpha]^{20}_D$ +3.7° (*c* 5%, in pyridine).

Anal. Calcd. for $C_{44}H_{37}O_2N_3$: C, 82.6; H, 5.8; N, 6.6. Found: C, 82.4; H, 5.7; N, 6.4.

By acidifying the above filtrate with acetic acid in the cold, α-monotrityl-L-histidine (III) precipitated together with a small amount of the ditrityl derivative and was purified by recrystallization from absolute alcohol; yield 0.6 g., m.p. 202°, $[\alpha]^{20}_D$ +23.7° (*c* 3.3%, in pyridine).

Anal. Calcd. for $C_{25}H_{23}O_2N_3$: C, 75.6; H, 5.8; N, 10.6. Found: C, 75.3; H, 5.6; N, 10.4.

N'-Monotrityl-L-histidine Methyl Ester Hydrochloride (V).—L-Histidine methyl ester dihydrochloride (2.4 g., 0.01 mole) was tritylated in the usual manner[5,6] in chloroform solution with 5.6 g. (0.02 mole) of trityl chloride in the presence of 6 ml. of triethylamine. To a solution of the resulting sirupy ditrityl derivative in dry ether was added 10 ml. of dry ether saturated with hydrogen chloride. The precipitated amorphous hydrochloride IV was filtered off, washed with dry ether and then dissolved in 20 ml. of absolute methanol. The solution was heated on the steam-bath for 2–3 minutes. On adding dry ether, V crystallized out; yield 2.7 g. (60%). The latter was purified by dissolving in water followed immediately by adding sodium hydrogen carbonate solution and extracting the free base with chloroform. The chloroform solution was washed with water and dried over sodium sulfate. Then, a few ml. of ether saturated with hydrogen chloride was added and the mixture evaporated to dryness *in vacuo*. The residue was dissolved in absolute methanol; on adding ether pure V crystallized out; m.p. 147°.

Anal. Calcd. for $C_{26}H_{26}O_2N_3Cl$: N, 9.4; Cl, 7.9. Found: N, 9.3; Cl, 7.8.

A few minutes after dissolution in methanol (*c* 5), the substance shows $[\alpha]^{20}_D$ +28.5°. On standing at room temperature for some 15 hours, this value drops to $[\alpha]_D$ +14° and some triphenyl methyl ether (m.p. 82°) is formed. On warming a water solution of V, triphenylcarbinol separates in almost quantitative yield.

O-Trityl-L-tyrosine Benzyl Ester Hydrochloride (VI).—To the solution of L-tyrosine benzyl ester *p*-toluenesulfonate[10] (4.4 g., 0.01 mole) in chloroform were added 4.2 ml. of triethylamine and 5.6 g. (0.02 mole) trityl chloride. After standing for 24 hours at room temperature, the solution was washed with water, dried over sodium sulfate and evaporated to dryness. The sirupy O,N-ditrityl derivative was monodetritylated in exactly the same way as the ditrityl-histidine ester. The yield of VI was 4.0 g. (72%); needles, m.p. 203–205° (after recrystallization from methanol–ether), $[\alpha]^{26}_D$ −24.2° (*c* 6.6%, in methanol).

Anal. Calcd. for $C_{35}H_{32}O_3NCl$: C, 76.4; H, 5.9; N, 2.5; Cl, 6.5. Found: C, 76.2; H, 5.8; N, 2.5; Cl, 6.7.

L-Phenylalanyl-L-leucine Benzyl Ester Hydrochloride.—A tetrahydrofuran solution of trityl-L-phenylalanine (4.1 g., 0.01 mole) and L-leucine benzyl ester (2.2 g., 0.01 mole) was prepared either by dissolving these substances in the solvent or by mixing solutions containing equivalent amounts of trityl-L-phenylalanine diethylammonium salt (4.8 g.) and L-leucine benzyl ester hydrochloride (2.6 g.) and filtering off the diethylammonium chloride. Then N,N'-dicyclohexylcarbodiimide (2.1 g., 0.01 mole) was added and the mixture was kept for 24 hours at room temperature. The filtrate of the N,N'-dicyclohexylurea (2.0 g., m.p. above 230°) was evaporated to dryness. The residue was dissolved in ethyl acetate-ether (1:1), washed successively with 5% acetic acid, three times with 2% sodium hydroxide, and finally with water, dried over sodium sulfate and evaporated to dryness. The sirupy residue (trityl-L-phenylalanyl-L-leucine benzyl ester) was detritylated by dissolving in 10 ml. of *N* methanolic hydrogen chloride and boiling the solution for 2 minutes. Upon evaporation to dryness, adding dry ether and cooling, the above hydrochloride precipitated; the product was recrystallized from methanol-ether; yield 1.7 g. (43%), needles, m.p. 161°.

(9) G. Amiard, R. Heymes and L. Velluz, *Bull. soc. chim. France*, 190 (1955).

(10) L. Zervas, M. Winitz and J. P. Greenstein, *J. Org. Chem.*, **22**, 1515 (1957).

Anal. Calcd. for $C_{22}H_{29}O_3N_2Cl$: C, 65.2; H, 7.2; N, 6.9; Cl, 8.8. Found: C, 65.1; H, 7.3; N, 7.0; Cl, 8.6.

L-Phenylalanyl-L-leucine.—A water solution of the above ester hydrochloride (2.0 g., 0.005 mole) was hydrogenated as usual in the presence of palladium black as a catalyst. Upon concentrating to a volume of 20 ml. and adjusting the *p*H to 6.2, the peptide precipitated in form of needles; yield 1.2 g. (88%), m.p. 258–260° (reported[11] for D-phenylalanyl-D-leucine 262°), $[\alpha]^{20}D$ +4.5° (*c* 9.3%, in 0.3 *N* hydrochloric acid).

Anal. Calcd. for $C_{15}H_{22}O_3N_2$: C, 64.7; H, 7.9; N, 10.0. Found: C, 64.05; H, 7.8; N, 10.1.

L-Glutaminyl-L-leucine.—Using N-trityl-L-glutamine diethylammonium salt (4.6 g., 0.01 mole) as the starting material, N-trityl-L-glutaminyl-L-leucine benzyl ester was prepared in the same manner as was described for the phenylalanine derivative. The sirupy coupling product was detritylated[5] and debenzylated in one operation by hydrogenation in water–methanol (1:5) solution with palladium black as a catalyst. Part of the triphenylmethane precipitated during the hydrogenation and was removed together with the catalyst by filtration. The filtrate was evaporated to dryness *in vacuo*. Upon adding acetone, the free peptide precipitated while the triphenylmethane remained in solution. The peptide was recrystallized from water–alcohol; yield 0.8 g. (31%), m.p. 204–205°, $[\alpha]^{20}D$ −34.4° (*c* 2.6%, in 0.1 *N* hydrochloric acid).

Anal. Calcd. for $C_{11}H_{21}O_4N_3$: C, 59.5; H, 8.2; N, 16.2. Found: C, 59.4; H, 8.3; N, 16.3.

N-Trityl-L-asparaginylglycine Ethyl Ester.—To a solution of 1.4 g. (0.01 mole) of glycine ethyl ester hydrochloride in 25 ml. of anhydrous pyridine, cooled to 0°, was added dropwise 0.45 ml. of phosphorus trichloride, with cooling and vigorous shaking. After 3 minutes, 3.7 g. (0.01 mole) of trityl-L-asparagine was added and the solution kept at room temperature for 10 minutes and then at 80° for 3–4 hours. The solution was then poured into 250 ml. of cold 10% acetic acid and extracted three times with ether. The ether layer was washed successively with dilute acetic acid, potassium hydrogen carbonate and water, dried over sodium sulfate, and finally concentrated to 40 ml. On cooling, 1 g. (22%) of product was obtained; needles, m.p. 182°, $[\alpha]^{26}D$ −67.9° (*c* 5.7%, in chloroform).

Anal. Calcd. for $C_{27}H_{29}O_4N_3$: C, 70.6; H, 6.3; N, 9.1. Found: C, 70.5; H, 6.4; N, 9.2.

L-Asparaginylglycine.—To a suspension of 2.3 g. (0.005 mole) of the above ester in 5 ml. of ethanol was added 5.5 ml. of *N* sodium hydroxide. After 15 minutes, the solution was diluted with water and then acidified with acetic acid. The precipitate (trityl-L-asparaginylglycine, m.p. 205–207°) was detritylated with acetic acid as described[5] to yield 0.72 g. (80%) of free peptide; prisms, m.p. 215–217°, $[\alpha]^{20}D$ +54.2° (*c* 5%, in water).

Anal. Calcd. for $C_6H_{11}O_4N_3$: C, 38.4; H, 5.8; N, 22.2. Found: C, 38.5; H, 6.0; N, 22.1.

N-Tritylglycyl-L-phenylalanylglycine Ethyl Ester.—To a solution of 4.6 g. (0.01 mole) of tritylglycyl-L-phenylalanine[5] and 1.03 g. of glycine ethyl ester, in 30 ml. of tetrahydrofuran, was added 2.2 g. of N,N′-dicyclohexylcarbodiimide. The urea derivative began to precipitate within a few minutes and was filtered off (2.2 g.) after the solution had been left at room temperature for 12 hours. The filtrate was evaporated to dryness, the residue dissolved in ethyl acetate and the solution washed successively with 5% sodium carbonate, dilute acetic acid, sodium hydrogen carbonate and water, dried over sodium sulfate and evaporated to dryness. Ether was added and 4.4 g. (80%) of product obtained from the cooled solution; prisms, m.p. 140°. This same product, when prepared previously by the mixed anhydride procedure, was obtained as a sirup.[5]

Anal. Calcd. for $C_{34}H_{35}O_4N_3$: C, 74.3; H, 6.4; N, 7.6. Found: C, 74.5; H, 6.5; N, 7.7.

Glycyl-L-phenylalanylglycine.—The above product was saponified and then detritylated with acetic acid, as described,[5] to yield 1.1 g. (80%) of the free peptide with $[\alpha]D$ +15.7° (*c* 5.6%, 0.2 *N* hydrochloric acid).

Anal. Calcd. for $C_{13}H_{17}O_4N_3$: N, 14.9; NH_2/N, 5.0. Found: N, 14.9; NH_2/N, 5.2.

The specific rotation observed (+15.7°) is exactly one-half that which was previously reported for the peptide prepared by the mixed anhydride procedure.[5] In order to ascertain whether racemization had occurred or if an error was involved we have repeated our former experiment[5] and we have found $[\alpha]D$ +15.5°. Furthermore, we have prepared the peptide by a third method, namely, by the carbobenzoxy method. Carbobenzoxyglycyl-L-phenylalanylglycine ethyl ester[12] (m.p. 117–118°, $[\alpha]D$ −12.4° in ethanol) yielded on saponification carbobenzoxyglycyl-L-phenylalanylglycine, m.p. 168°, and after hydrogenation the free peptide, which exhibited $[\alpha]D$ +15.5°. Both these values are identical with that reported here and we must therefore conclude that the previously reported[5] value of $[\alpha]D$ +30.9° was in error.

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ATHENS, GREECE

[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

Hypotensive Agents. I. The Effect of Hydrogen Bonding in Some 4-Dialkylaminoalkylaminoquinolines

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The reaction of a variety of 5-chloro- and 7-chloro-4-dialkylaminoalkylaminoquinolines with 2-chlorobenzyl chloride has been investigated. In many instances the use of one equivalent of the alkylating agent gave a 1-(2-chlorobenzyl)-quinoline derivative. Evidence for intramolecular hydrogen bonding in many of these 4-dialkylaminoalkylaminoquinolines as well as in the corresponding 1-(2-chlorobenzyl)-quinolinium derivatives is presented. This phenomenon appears to be responsible for the marked steric hindrance associated with the terminal nitrogen atom in the side chain and for the ease of hydrolysis of the 1-quinolinium derivative to the corresponding 4-quinolones.

In the course of our continuing investigation of quinoline chemistry we have prepared some mono- and bis-quaternary salts of a variety of 4-dialkylaminoalkylaminoquinolines. Most of these compounds as well as their pharmacology will be reported in other papers. The present communication deals mainly with some of our observations using 2-chlorobenzyl chloride as the quaternizing agent.

From the reaction of one equivalent of 2-chlorobenzyl chloride with 7-chloro-4-(2-diethylaminoethylamino)-quinoline (Ib)[1] (see Chart I) in acetonitrile a solid product was obtained which gave the correct analysis for a monoquaternary salt. When this material, which was soluble in water to only about one per cent., was dissolved in

(1) The 5-chloro compounds are designated by a and the 7-chloro compounds by b.