New Methods in Peptide Synthesis. Part V.[1] On α- and γ-Diphenylmethyl and Phenacyl Esters of L-Glutamic Acid[2]

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Several diphenylmethyl and phenacyl esters of monocarboxylic acids (L-proline, *S*-trityl-L-cysteine, L-glutamine, and L-asparagine) as well as α- and γ-esters of L-glutamic acid are described. The structures of the α-and γ-esters have been established by conversion of the α-esters into derivatives of L-glutamine. All these esters should prove to be useful in peptide synthesis, since the carboxy protecting groups can be selectively removed; the diphenylmethyl group by hydrogen chloride in certain non-polar solvents, and the phenacyl group by the action of sodium phenyl sulphide. Furthermore, both protecting groups can be removed by hydrogenolysis. An explanation for the unexpected hydrogenolysis of the phenacyl esters is presented.

We have been concerned with the use of the diphenylmethyl and phenacyl groups for the temporary protection of the carboxy-group of amino-acids in peptide synthesis since both groups can be selectively removed by specific reagents.[3,4] Indeed, many peptide syntheses have been reported using diphenylmethyl and phenacyl esters of amino-acids as intermediates.[4] The preparation of some new esters is reported, *i.e.*, the diphenylmethyl ester of L-glutamine, and the phenacyl esters of L-proline and *S*-trityl-L-cysteine.

The carboxy protection is of special interest for dicarboxylic amino-acids, since α- and ω-esters are used for the specific synthesis of ω- and α-peptides, respectively. Here we deal with L-glutamic acid and its diphenylmethyl and phenacyl esters. The α-esters were prepared by direct esterification, whereas the γ-esters were prepared indirectly. Thus, using the method of Aboderrin, Delpierre, and Fruton,[5] L-glutamic acid naphthalene-2-sulphonate with 1 mol. of diphenyl

[1] Part IV, I. Phocas, C. Yovanidis, I. Photaki, and L. Zervas, *J. Chem. Soc.* (*C*), 1967, 1506.

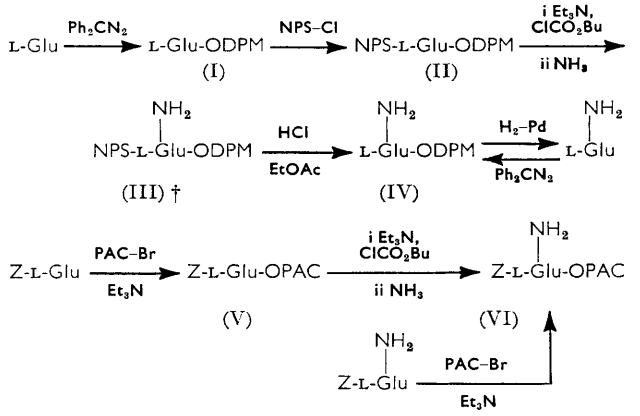
[2] Presented in part at the Eighth European Peptide Symposium: L. Zervas, I. Photaki, C. Yovanidis, J. Taylor, I. Phocas, and V. Bardakos, "Peptides: Proceedings of the Eighth European Symposium," Noordwijk, The Netherlands, 1966, ed. H. C. Beyerman, North-Holland Publishing Co., Amsterdam, 1967, p. 28.

[3] J. C. Sheehan and G. D. Daves, jun., *J. Org. Chem.*, 1964, **29**, 2006.

[4] G. C. Stelakatos, A. Paganou, and L. Zervas, *J. Chem. Soc.*, 1966, 1191; cf. also R. G. Hiskey and J. B. Adams, *J. Amer. Chem. Soc.*, 1965, **87**, 3969.

[5] (*a*) A. A. Aboderrin, G. R. Delpierre, and J. S. Fruton, *J. Amer. Chem. Soc.*, 1965, **87**, 5469; (*b*) "Peptides: Proceedings of the Fifth European Symposium," Oxford, 1962, ed. G. T. Young, Pergamon Press, Oxford, 1963, p. 261.

diazomethane, gave the expected α-diphenylmethyl ester (I).[5] Similarly, the action of equimolar proportions of phenacyl bromide and triethylamine on *N*-benzyloxycarbonyl L-glutamic acid yields the *N*-protected α-phenacyl ester (V) as would be expected, knowing that



SCHEME 1 *

DPM = Ph_2CH, PAC = $BzCH_2$, NPS = o-NO_2·C_6H_4S,
Z = Ph·CH_2·O·CO, Bu = Me_2CH·CH_2

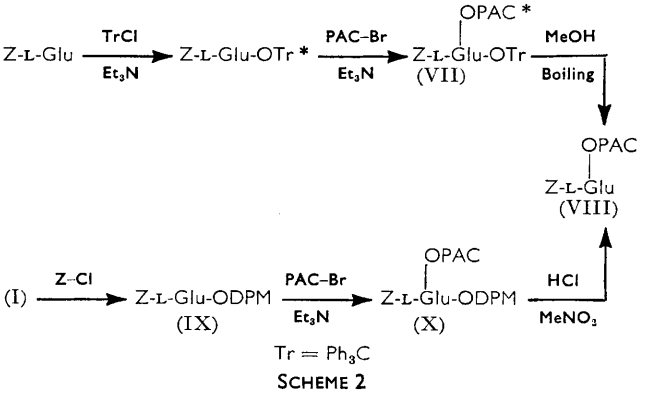
* Here and elsewhere the abbreviations used for protecting groups and amino-acid residues are those recommended by the committee on Nomenclature of the Fifth European Peptide Symposium.[5a]
† Compound not isolated.

N-acyl L-glutamic acids are preferentially esterified in the α-carboxy-group by the action of alkylating agents.[6] The structure of both α-esters was confirmed by their conversion into L-glutamine or a derivative by the series of reactions illustrated in Scheme 1. Thus, the α-diphenylmethyl ester was converted into its *N*-*o*-nitrophenylsulphenyl derivative (II) which was coupled with ammonia to yield *N*-*o*-nitrophenylsulphenyl-L-glutamine diphenylmethyl ester (III). Removal of the *o*-nitrophenylsulphenyl group from (III) gave L-glutamine diphenylmethyl ester (IV) which was identical to the ester prepared by direct esterification of L-glutamine naphthalene-2-sulphonate with diphenyldiazomethane. Furthermore, hydrogenation of the ester (IV) obtained from the α-diphenylmethyl ester of L-glutamic acid yielded L-glutamine. Similarly, the *N*-benzyloxycarbonyl-L-glutamic acid α-phenacyl ester (V) was converted to *N*-benzyloxycarbonyl-L-glutamine phenacyl ester (VI), which was identical to the compound prepared by the reaction of phenacyl bromide with *N*-benzyloxycarbonyl-L-glutamine.

The *N*-benzyloxycarbonyl derivative (VIII) of the γ-phenacyl ester of L-glutamic acid has been prepared by two methods which are outlined in Scheme 2. The first method is dependent on the preferential esterification of *N*-benzyloxycarbonyl-L-glutamic acid in the α-position;[6] the *N*-protected amino-acid is first treated with triphenylmethyl chloride and then with phenacyl bromide. The *N*-protected α-trityl γ-phenacyl diester (VII) which is presumably formed was not isolated but

[6] G. H. L. Nefkens and R. J. F. Nivard, *Rec. Trav. chim.*, 1964, **83**, 199.

was boiled with methanol, a procedure known to split the trityl ester bond.[4,7] The resulting *N*-benzyloxycarbonyl-L-glutamic acid γ-ester (VIII) was isolated in pure form by t.l.c. It was identical to the compound synthesised from the α-diphenylmethyl ester of L-glutamic acid by the series of reactions shown in Scheme 2

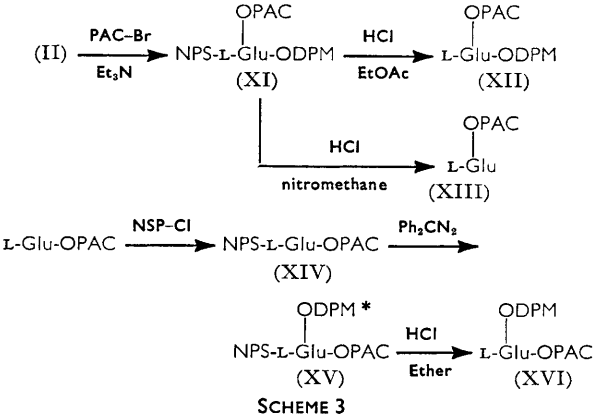


SCHEME 2

* Compound not isolated.

thus proving it to be a γ-ester. In this Scheme, the α-diphenylmethyl ester is converted first to the *N*-benzyloxycarbonyl derivative (IX) and then esterified with phenacyl bromide in the γ-position to give a mixed diester of the protected amino-acid (X) which can be converted to the γ-ester by differential scission of the diphenylmethyl group with hydrogen chloride in nitromethane.

Furthermore, the mixed diesters, L-glutamic acid α-diphenylmethyl γ-phenacyl ester (XII) and L-glutamic acid α-phenacyl γ-diphenylmethyl ester (XVI), were prepared from *N*-*o*-nitrophenylsulphenyl-L-glutamic acid α-diphenylmethyl ester (II) and *N*-*o*-nitrophenylsulphenyl-L-glutamic acid α-phenacyl ester (XIV) respectively (Scheme 3). Thus, compound (II) was



SCHEME 3

* Compound not isolated.

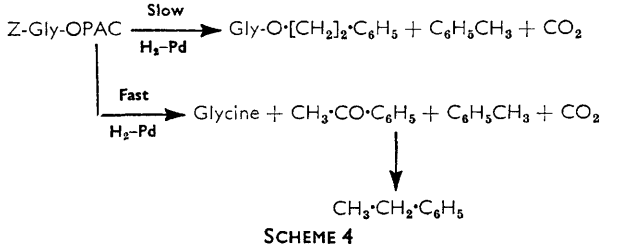
esterified with phenacyl bromide, and compound (XIV) with diphenyldiazomethane, to give the *N*-protected

[7] E. Gazis, B. Bezas, G. C. Stelakatos, and L. Zervas, "Peptides: Proceedings of the Fifth European Symposium," Oxford, 1962, ed. G. T. Young, Pergamon Press, Oxford, 1963, p. 17.

diesters (XI) and (XV), respectively; these gave the hydrochlorides of the diesters (XII) and (XVI) by the action of hydrogen chloride in ether or ethyl acetate. Finally, treatment of compound (XI) with hydrogen chloride in nitromethane gave the free γ-phenacyl ester of L-glutamic acid (XIII). As discussed in a previous Paper,[4] the cleavage of the diphenylmethyl ester group proceeds much more slowly in ethyl acetate than in nitromethane so that the *N*-protected diester (XI) can be transformed selectively to either the free diester (XII) or to the free monoester (XIII), depending on the solvent used.

Previously,[4] we have reported that phenacyl esters can be deblocked by hydrogenolysis in the presence of a palladium catalyst at s.t.p., yielding the free acids and ethylbenzene. This reaction is somewhat unexpected since it is known that, normally, only benzyl alcohol and its α-substituted compounds (and their *O*-derivatives) are hydrogenolysed under these conditions,[8] the most striking applications of this finding being the benzyloxycarbonyl method in peptide synthesis,[9a] and the use of benzyl esters of phosphoric acid for phosphorylation purposes.[9b,c] To our knowledge, no evidence for the hydrogenolysis of β-phenethyl alcohol and its *O*-derivatives under the above conditions has been reported.

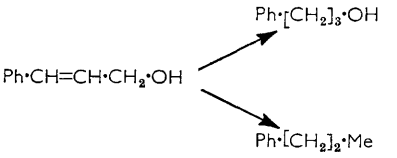
A close examination of the hydrogenolysis of phenacyl esters showed that, although the consumption of hydrogen was always high, the yields of free acids were never greater than 70—75%. The explanation for this seems to be that two reactions occur simultaneously during hydrogenation (Scheme 4). The phenacyl ester rapidly



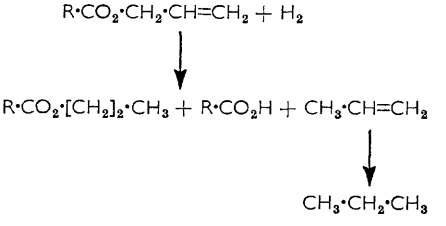
SCHEME 4

undergoes hydrogenolysis to the free acid and acetophenone, which is further reduced to ethylbenzene. However, small amounts of the ester which do not react by this route are hydrogenated at the keto-group to form β-phenethyl ester; this does not undergo hydrogenolysis to the free acid, the yields of which are, consequently, reduced. It has been shown that glycine β-phenethyl ester (prepared synthetically) does not undergo hydrogenolysis with a palladium catalyst; further, the presence of the ester can be chromatographically demonstrated after catalytic hydrogenation of *N*-benzyloxycarbonyl glycine phenacyl ester.

This reaction of the phenacyl esters * parallels the behaviour of cinnamyl alcohol during hydrogenolysis, where a mixture of n-propylbenzene and 3-phenylpropan-1-ol is formed probably by a competing reaction and not by successive reactions [8,10a]



Thus, it seems that the phenacyl esters and cinnamyl alcohol behave like benzyl esters during hydrogenolysis because, due to the presence of the carbonyl group in the phenacyl esters, and the double bond in cinnamyl alcohol, the conjugated double bond system of the benzene ring is extended to the vicinity of the ester or alcohol group. Even the presence of an isolated double bond in the β-position, as in allylic alcohols and their esters, causes hydrogenolysis to proceed in a similar manner, and a mixture of products is obtained.[10b]



EXPERIMENTAL

Anhydrous solvents were used for the esterification of amino-acids and for the cleavage of esters. Removal of solvents by evaporation under reduced pressure was carried out at 35—40° unless otherwise stated. Before analysis, substances were dried over P_2O_5 in high vacuum at room temperature unless another temperature is indicated; microanalyses were by Dr. H. Mantzos, Analytical Laboratory of the Royal Hellenic Research Foundation. All substances were pure as demonstrated by t.l.c. [11] on Kieselgel G in one of the following systems, (*A*) butanol–acetic acid–water–pyridine (30 : 6 : 24 : 20), (*B*) butanol–acetic acid–water, (100 : 10 : 30), (*C*) chloroform–carbon tetrachloride–methanol (6 : 3 : 1), (*D*) benzene–pyridine–acetic acid (35 : 3 : 0·5), (*E*) toluene–pyridine–acetic acid (80 : 10 : 1), (*F*) isopentyl alcohol–methanol–water (10 : 10 : 10). Spots were demonstrated by ninhydrin where the substance contained a free amino-group and by iodine for *N*-protected amino-acids.

Naphthalene-2-sulphonic Acid Salts.—The naphthalene-2-sulphonic acid salt of L-glutamic acid was prepared by the general procedure already reported,[5] the crude *product* being recrystallised from acetone (yield 77%), m. p. 169—171° (Found: N, 3·9; S, 8·9. $C_{15}H_{17}NO_7S$ requires N, 3·95; S, 9·0%).

* On reduction of phenacyl alcohol with sodium amalgam, a mixture of compounds is also formed, namely phenyl glycol and phenyl methyl carbinol, cf. Beilstein, 4th edn., 1925, vol. VIII, p. 90.

[8] W. H. Hartung and R. Simonoff, *Org. Reactions*, 1953, **7**, 263, 267.

[9] (*a*) M. Bergmann and L. Zervas, *Ber.*, 1932, **65**, 1192; Ger.P. 556,798/1932; (*b*) L. Zervas, *Naturwiss.*, 1939, **27**, 317; (*c*) F. R. Atherton, H. T. Openshaw, and A. R. Todd, *J. Chem. Soc.*, 1945, 382, 660.

[10] (*a*) R. Baltzly and J. S. Buck, *J. Amer. Chem. Soc.*, 1943, **65**, 1984; (*b*) A. Eiger, M. Fetizon, J. Henniker, and L. Jacque, *Compt. rend.*, 1960, **251**, 2194.

[11] M. Brenner and A. Niederwieser, *Experientia*, 1960, **16**, 378.

The corresponding salt of L-glutamine was precipitated from an aqueous solution of L-glutamine (2·92 g., 0·02 mole) and naphthalene-2-sulphonic acid (4·72 g., 0·021 mole) by acetone and ether. The *compound* (6·1 g., 86%) had m. p. >230° (Found: N, 7·9; S, 9·15. $C_{15}H_{18}N_2O_6S$ requires N, 7·9; S, 9·05%).

L-*Asparagine naphthalene-2-sulphonate.* This was prepared in the same way as the corresponding salt of L-glutamine. L-Asparagine monohydrate (15 g., 0·1 mole) gave the *salt* (28 g., 87%), m. p. >230° (Found: N, 8·05; S, 9·5. $C_{14}H_{16}N_2O_6S$ requires N, 8·2; S, 9·4%).

L-*Glutamic Acid α-Diphenylmethyl Ester* (I).—The naphthalene-2-sulphonate salt of L-glutamic acid prepared from the amino-acid (2·94 g., 0·02 mole) and naphthalene-2-sulphonic acid monohydrate (4·52 g., 0·02 mole) was dissolved in dimethylformamide (10 ml.). A solution of diphenyldiazomethane (4·0 g., 0·02 mole) in dimethylformamide (10 ml.) was added dropwise during 15—20 min. to the solution which was stirred and kept at 50°. After addition of the diazomethane, the reaction mixture was cooled to room temperature and sodium acetate (5 g.) in water (20 ml.) was added. The solution was scratched and cooled, when the free *ester* (I) crystallised out and was filtered off and washed successively with water, ethanol, and ether.

The product (4·2 g., 67%) had m. p. 162·5—163·5°; after the product had been boiled briefly with ethanol the m. p. was raised to 165°; $[\alpha]_D^{20}$ −5·0° (*c* 2·0 in methanol containing the equivalent amount of β-naphthalene sulphonic acid) (Found: C, 68·95; H, 5·9; N, 4·3. $C_{18}H_{19}NO_4$ requires C, 69·0; H, 6·1; N, 4·5%).

L-*Asparagine Diphenylmethyl Ester Naphthalene-2-sulphonate.*—A solution of L-asparagine naphthalene-2-sulphonate (5·1 g., 0·015 mole) in dimethylformamide (10 ml.) was heated to 50° and a solution of diphenyldiazomethane (4·4 g., 0·023 mole) in dimethylformamide (10 ml.) was added during 2 min. The mixture was stirred at 50° for 15 min. and then cooled to room temperature before the addition of ether. The mixture was scratched and cooled for several hours in a refrigerator, and gave an amorphous precipitate of the amino-acid ester salt. The supernatant liquid was decanted off and the precipitate washed several times with ether and then recrystallised from isopropyl alcohol. The material obtained was washed with ether to give the *naphthalene-2-sulphonate* (5·4 g., 69%) m. p. 143—144° (from isopropyl alcohol), $[\alpha]_D^{24}$ +4·8° (*c* 6·0 in dimethylformamide) (Found: C, 62·8; H, 5·2; N, 5·5. $C_{27}H_{26}N_2O_6S,0{\cdot}5H_2O$ requires C, 62·9; H, 5·2; N, 5·4%). The ester was converted to the corresponding hydrochloride which showed the same m. p. (155°) and chromatographic behaviour as the material prepared by a different route.[4]

N-*Benzyloxycarbonyl-L-proline Phenacyl Ester.*—This was prepared from *N*-benzyloxycarbonyl-L-proline (0·01 mole) and phenacyl bromide (0·01 mole) in the same manner as described for the corresponding derivative of glycine.[4] The yield was 83%, m. p. 93—94°, $[\alpha]_D^{15}$ −76·7° (*c* 4 in dimethylformamide) (Found: C, 68·8; H, 5·95; N, 3·7. $C_{21}H_{21}NO_5$ requires C, 68·7; H, 5·8; N, 3·8%).

L-*Proline Phenacyl Ester Hydrobromide.*—This was prepared from the above *N*-benzyloxycarbonyl derivative as described for the corresponding derivative of glycine.[4] Yield 82%, m. p. 157—158°, $[\alpha]_D^{15}$ −49° (*c* 4 in dimethylformamide) (Found: C, 49·6; H, 5·15; Br, 25·35; N, 4·3. $C_{13}H_{15}NO_3,HBr$ requires C, 49·7; H, 5·1; Br, 25·4; N, 4·45%).

N-o-*Nitrophenylsulphenyl-S-trityl-L-cysteine Phenacyl Ester.*—To a solution of *N-o*-nitrophenylsulphenyl-*S*-trityl-L-cysteine (2·1 g., 0·004 mole) in ethyl acetate (20 ml.), cooled to 0°, phenacyl bromide (0·8 g., 0·004 mole) and triethylamine (0·56 ml. 0·004 mole) were added and the mixture was set aside for 12 hr. at room temperature. The mixture was then diluted with ethyl acetate and washed successively with water, sulphuric acid, potassium hydrogen carbonate, and water, and then dried (Na_2SO_4), and evaporated to dryness under reduced pressure. Upon the addition of methanol and after being set aside at room temperature, the pure N-o-*nitrophenylsulphenyl ester* crystallised out (0·9 g., 76%), m. p. 115°, $[\alpha]_D^{15}$ −46·2° (*c* 2 in chloroform) (Found: C, 68·3; H, 4·8; N, 4·6; S, 9·8. $C_{36}H_{30}N_2O_5S_2$ requires C, 68·3; H, 4·8; N, 4·6; S, 9·8%).

S-*Trityl-L-cysteine Phenacyl Ester Hydrochloride.*—To a solution of the above *N-o*-nitrophenylsulphenyl ester (1·3 g., 0·002 mole) and triphenylmethyl chloride (0·8 g.) in ether (14 ml.), a few ml. of ether saturated with hydrogen chloride were added. The precipitate which formed was washed with ether and crystallised from ethyl acetate–ether yielding the *hydrochloride* (yield 50%), m. p. 68° (Found: Cl, 6·8; N, 2·7; S, 6·2. $C_{30}H_{27}NO_3S,HCl$ requires Cl, 6·8; N, 2·7; S, 6·2%).

N-o-*Nitrophenylsulphenyl-L-glutamic Acid α-Diphenylmethyl Ester* (II).—This was obtained as the dicyclohexylammonium salt from *o*-nitrophenylsulphenyl chloride and L-glutamic acid α-diphenylmethyl ester by procedure B described earlier.[12] With 0·005 molar quantities, the yield of the *dicyclohexylammonium salt of* (II) was 65%, m. p. 180—181 (from large volume of ethanol) $[\alpha]_D^{19}$ −33° (*c* 1 in methanol) (Found: C, 66·8; H, 7·1; N, 6·6. $C_{36}H_{45}N_3O_6S$ requires C, 66·8; H, 7·0; N, 6·5%).

L-*Glutamine Diphenylmethyl Ester* (IV).—(*a*) The naphthalene-2-sulphonate salt of L-glutamine (1·77 g., 0·005 mole) was esterified by diphenyldiazomethane (1·46 g., 0·0075 mole) in dimethylformamide (12 ml.) at 50° by the procedure already reported.[5] The reaction mixture was cooled and ether was added; the crude amino-acid salt which separated out (2·1 g., 78%), was converted to the hydrochloride as follows. A sample (1·7 g.) of the crude product was suspended in methanol (2 ml.) and ethyl acetate (5 ml.) and shaken with a dilute solution of sodium hydrogen carbonate. The aqueous layer was discarded and the organic layer thoroughly washed with water until the washings were no longer alkaline. After drying (K_2CO_3), hydrochloric acid in ether was added. A crystalline precipitate of the *hydrochloride* was formed (0·3 g., 25%), m. p. 169° (from methanol–ethyl acetate), $[\alpha]_D^{22}$ −7·5° (*c* 3 in dimethylformamide) (Found: C, 62·35; H, 6·1; N, 8·1. $C_{18}H_{20}N_2O_3,HCl$ requires C, 62·0; H, 6·1; N, 8·0%).

(*b*) For the coupling reaction with ammonia, the dicyclohexylammonium salt of compound (II) (3·4 g., 0·005 mole) was converted to the free acid by shaking a suspension of the salt in ethyl acetate with dilute sulphuric acid; the organic layer was repeatedly washed with water, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was dissolved in tetrahydrofuran (12 ml.) and the solution cooled to 0° before the addition of triethylamine (0·7 ml., 0·005 mole) and isobutyl chloroformate (0·6 ml.). The mixture was shaken gently at this

[12] L. Zervas, D. Borovas, and E. Gazis, *J. Amer. Chem. Soc.*, 1963, **85**, 3660.

temperature before the addition of a solution of dry ammonia in dioxan (35 ml.). After 10 min. at room temperature, the solvent was removed under reduced pressure and the resulting oil dissolved in ethyl acetate. The ethyl acetate solution was washed successively with water, dilute sulphuric acid, water, sodium hydrogen carbonate, and again with water until the washings were no longer alkaline; the solution was dried (Na_2SO_4) and the ethyl acetate removed under reduced pressure. The oily *N-o*-nitrophenylsulphenyl-L-glutamine diphenylmethyl ester (III) thus formed was dissolved in ethyl acetate–ether. Upon the addition of hydrogen chloride in ether to the solution a gummy precipitate appeared which crystallised on being scratched and cooled, to give the hydrochloride of L-glutamine diphenylmethyl ester (1·1 g., 60%), m. p. 169° (from methanol–ethyl acetate) [mixed m. p. with material from (*a*) was undepressed], $[\alpha]_D^{22}$ −7·0° (*c* 3 in dimethylformamide) (Found: C, 61·9; H, 6·1; N, 8·3%).

Hydrogenation (Pd catalyst) of the above ester hydrochloride, [prepared by method (*b*)] in methanol containing triethylamine and a small amount of acetic acid yielded L-glutamine, m. p. 176—180° (lit.,[13a] m. p. 186°) (m. p. not raised from aqueous ethanol), $[\alpha]_D^{24}$ +6·4° (*c* 3 in water) {lit.,[13a] $[\alpha]_D^{25}$ +6·5° (*c* 2 in water)} (Found: N, 19·2. $C_5H_{10}N_2O_3$ requires N, 19·2%).

N-Benzyloxycarbonyl-L-glutamic Acid α-Phenacyl Ester (V).—*N*-Benzyloxycarbonyl-L-glutamic acid [8] (2·8 g., 0·01 mole) was dissolved in dimethylformamide (7 ml.) containing triethylamine (1·4 ml., 0·01 mole) and this was followed immediately by the addition of phenacyl bromide (2 g.). After being kept for 2 days at room temperature, ethyl acetate (100 ml.) was added and the resulting solution washed with water, dried (Na_2SO_4), and concentrated under reduced pressure to 50 ml. Dicyclohexylamine (4 ml.) was added, and after being cooled and filtered the *dicyclohexylammonium salt* of the above acid (V) was obtained, m. p. 149—151° (from ethanol), yield 54%; $[\alpha]_D^{14}$ −16·5° (*c* 3 in methanol) (Found: C, 67·5; H, 7·8; N, 4·8. $C_{33}H_{44}N_2O_7$ requires C, 68·2; H, 7·6; N, 4·8%). The above salt (1 g.) was converted to the free acid by treatment with dilute sulphuric acid and extraction into ethyl acetate–ether. The solvents were evaporated off to leave a syrupy residue which was dissolved in acetone (5 ml.) from which the pure *α-phenacyl ester* (V) was precipitated by the dropwise addition of water (0·6 g., 87%), m. p. 69—79° (after drying over P_2O_5 as substance is hygroscopic), $[\alpha]_D^{21}$ −31·4° (*c* 1·0 in methanol) (Found: C, 63·0; H, 5·5; N, 3·5. $C_{21}H_{21}NO_7$ requires C, 63·2; H, 5·3; N, 3·5%).

N-Benzyloxycarbonyl-L-glutamine Phenacyl Ester (VI).—(*a*) A solution of *N*-benzyloxycarbonyl-L-glutamine (2·8 g., 0·01 mole) and triethylamine (1·4 ml.) in ethanol (15 ml.) was cooled to 0° and phenacyl bromide (2·0 g., 0·01 mole) was added. After 12 hr. at room temperature, a crystalline substance appeared which was redissolved by warming the solution on a water-bath and by the addition of ethanol (10 ml.) and triethylamine (0·5 ml.). The mixture was cooled and crystals of the *ester* (VI) were obtained (3·2 g., 80%), m. p. 151—152° (unchanged from ethanol), $[\alpha]_D^{22}$ −18·3° (*c* 3 in dimethylformamide) (Found: C, 63·3; H, 5·8; N, 7·2. $C_{21}H_{22}N_2O_6$ requires C, 63·3; H, 5·6; N, 7·0%).

(*b*) Compound (V) (0·8 g., 0·002 mole) was dissolved in tetrahydrofuran (8 ml.) and the solution cooled to −3° before the addition of triethylamine (0·28 ml.) and isobutyl chloroformate (0·26 ml.). After 15 min. at −3°, a solution of dry ammonia in tetrahydrofuran was added to the mixture which was then set aside at room temperature for 90 min. The solvent was removed under reduced pressure and the crystalline residue was taken up in ethyl acetate (50 ml.). The resulting solution was washed with water (×3), dried (Na_2SO_4), and then concentrated under reduced pressure until the product began to crystallise out. Light petroleum was added to the mixture which, after being cooled in the refrigerator, gave the ester (VI) (0·56 g.), m. p. 151—152° (from ethanol), $[\alpha]_D^{22}$ −18·3° (*c* 3 in dimethylformamide) (Found: C, 63·3; H, 5·6; N, 7·2%).

L-Glutamic Acid α-Phenacyl Ester Hydrobromide.—To a solution of the ester (V) (1·2 g., 0·003 mole) in ethyl acetate (15 ml.), a solution of hydrogen bromide in acetic acid (8N; 3·5 ml.) was added. After 1 hr. at room temperature, the solution was taken to dryness under reduced pressure at 25° to remove the excess of hydrogen bromide; more ethyl acetate was then added. The undissolved crystalline product was filtered off, washed with ethyl acetate and ether, to give the *α-phenacyl ester hydrobromide* (0·99 g., 96%), m. p. 163—164° (from ethyl acetate containing a small quantity of ethanol), $[\alpha]_D^{24}$ +23·3° (*c* 3 in methanol) (Found: C, 45·2; H, 4·6; Br, 23·45; N, 4·3. $C_{13}H_{15}NO_5$,HBr requires C, 45·1; H, 4·7; Br, 23·1; N, 4·0%).

N-Benzyloxycarbonyl-L-glutamic Acid α-Diphenylmethyl Ester (IX).—To a suspension of compound (I) (2·2 g., 0·007 mole) in dioxan (15 ml.) and water (7·5 ml.), sodium hydroxide (2N; 2 ml.) was added and the mixture stirred at 0°. Immediately afterwards and keeping the mixture well stirred at 0° and the pH at 7·0, benzyloxycarbonyl chloride (total 1·4 ml.) and sodium hydroxide (2N; 5·2 ml.) were added alternately in small portions during 40 min. The mixture was then diluted with water–dioxan (2 : 1), filtered, and the resulting solution washed with ether (×2) to remove unchanged benzyloxycarbonyl chloride. The water layer was then acidified with dilute sulphuric acid and extracted with ethyl acetate–ether. The organic layer was then washed with water until the washings were no longer acid to Congo Red paper, dried (Na_2SO_4), and evaporated to dryness. The resulting oil was dissolved in ethyl acetate (20 ml.) and upon the addition of cyclohexylamine (0·7 ml.), the *cyclohexylammonium salt of* (IX) was obtained (2·0 g., 54%), m. p. 176—177° (from ethanol), $[\alpha]_D^{20}$ −19·3° (*c* 1·5 in methanol) (Found: C, 69·95; H, 7·0; N, 5·3. $C_{32}H_{38}N_2O_6$ requires C, 70·2; H, 7·0; N, 5·1).

N-Benzyloxycarbonyl-L-glutamic Acid α-Diphenylmethyl γ-Phenacyl Ester (X).—The ester (IX) was liberated from the corresponding cyclohexylammonium salt (1·2 g., 0·0022 mole) as described for the preparation of compound (II) from its salt. It was then treated for 20 hr. at room temperature with phenacyl bromide (0·4 g., 0·002 mole) in the presence of triethylamine (0·28 ml., 0·002 mole) in absolute ethyl acetate (12 ml.). The triethylamine hydrobromide was filtered off and the solution was diluted with more ethyl acetate, and then washed with water, dilute sulphuric acid, again with water, and then dried (Na_2SO_4) and evaporated to dryness under reduced pressure. Upon removal of solvent the *diester* (X) was left as a crystalline residue (1·0 g.), m. p. 119—120°. Recrystallisation from methanol yielded the pure compound (0·75 g., 68%), m. p.

[13] (*a*) J. P. Greenstein and M. Winitz, "Chemistry of the Amino-acids," John Wiley, 1961, vol. 3, p. 1930; (*b*) p. 1819.

124·5—125·5°, $[\alpha]_D^{19}$ −22·8° (*c* 3 in dimethylformamide) (Found: C, 72·2; H, 5·7; N, 2·6. $C_{34}H_{31}NO_7$ requires C, 72·15; H, 5·5; N, 2·5).

N-*Benzyloxycarbonyl*-L-*glutamic Acid* γ-*Phenacyl Ester* (VIII).—(*a*) The benzyloxycarbonyl derivative of compound (X) (0·340 g., 0·0006 mole) was dissolved in nitromethane (6 ml.) and a solution of hydrogen chloride in nitromethane (2 ml.; 2N) was added. After 2 hr. at room temperature the nitromethane was removed under reduced pressure and replaced by ethyl acetate. The ethyl acetate solution was washed with water until it was no longer acid, dried (Na_2SO_4), and evaporated down to a volume of 5 ml. Upon the addition of dicyclohexylamine (0·12 ml.) a precipitate of the *dicyclohexylammonium salt* of (VIII) was formed (0·22 g., 60%), m. p. 141—142° (from isopropyl alcohol), $[\alpha]_D^{20}$ +7·7° (*c* 3 in methanol) (Found: C, 68·1; H, 7·8; N, 4·8. $C_{33}H_{44}N_2O_7$ requires C, 68·25; H, 7·6; N, 4·8%).

(*b*) *N*-Benzyloxycarbonyl-L-glutamic acid (2·81 g., 0·01 mole) was dissolved in tetrahydrofuran (12 ml.) containing triethylamine (1·4 ml.) and this was followed by the immediate addition of triphenylmethyl chloride (2·8 g., 0·01 mole). After 20 hr. at room temperature, the triethylamine hydrochloride was filtered off and phenacyl bromide (2 g., 0·01 mole) and triethylamine (1·4 ml.) were added. This solution was kept for 15 hr. at room temperature and the triethylamine hydrobromide formed was then filtrated off. The filtrate was evaporated to dryness under reduced pressure and the residue heated under reflux for 15 min. with methanol (30 ml.). Again the solvent was removed under reduced pressure and the residue taken up in ethyl acetate (30 ml.); dicyclohexylamine was then added. The first precipitate which appeared was filtered off (1·3 g., m. p. 154—155°, probably *N*-benzyloxycarbonyl L-glutamic acid bisdicyclohexylammonium salt) and the filtrate evaporated to dryness. The remaining material was dissolved in methanol; scratching and seeding precipitated trityl methyl ether, which was filtered off. Finally, the filtrate was evaporated to dryness under reduced pressure and the residue dissolved in absolute ethyl acetate. From this solution material (1·8 g.) m. p. 127—132° was slowly precipitated and was chromatographed using system (*D*). The slowest moving component was collected by extracting a suspension of the Kieselgel into ethyl acetate which was then washed with water, dried (Na_2SO_4), and evaporated to dryness. The resulting oil was dissolved in acetone from which the pure γ-*ester* (VIII) was precipitated as the *dicyclohexylammonium salt* (recovery 50%), m. p. 141—142°; $[\alpha]_D^{17}$ +7·0° (*c* 3 in methanol) (Found: C, 68·1; H, 7·6; N, 4·9%).

N-o-*Nitrophenylsulphenyl*-L-*glutamic Acid* α-*Phenacyl Ester* (XIV).—The hydrobromide of L-glutamic acid α-phenacyl ester (0·35 g., 0·001 mole) was dissolved in dioxan (7 ml.) and water (1 ml.) and the solution cooled to 0° before the addition of sodium hydroxide (N; 1 ml.). From this solution, the *N*-*o*-nitrophenylsulphenyl derivative of the above ester was prepared by procedure *B* reported earlier,[12] with the modification that the temperature of the reaction mixture was kept at 0° and the pH *ca.* 6·0. The ester (XIV) was isolated as its *dicyclohexylammonium salt* (0·41 g., 68%), m. p. 148—149° (unchanged from ethyl acetate), $[\alpha]_D^{29}$ −57·7° (*c* 3 in methanol) (Found: C, 61·8; H, 6·9; N, 7·2; S, 5·2. $C_{31}H_{41}N_3O_7S$ requires C, 62·1; H, 6·9; N, 7·0; S, 5·3%).

L-*Glutamic Acid* α-*Phenacyl* γ-*Diphenylmethyl Ester* (XVI).—The ester (XIV) [obtained from its dicyclohexylammonium salt (0·0009 mole) in the manner described for the preparation of compound (II) from its salt] was dissolved in tetrahydrofuran (10 ml.) and then diphenyldiazomethane (0·235 g., 0·001 mole) was added. The solution was warmed to 50° and kept at this temperature for 3 hr. The solvent was then removed under reduced pressure and the residue taken up in ethyl acetate–ether (1 : 1). The resulting solution was cooled, washed with cold 0·5N-sodium hydroxide (×2) and water, and then dried (Na_2SO_4) and evaporated to dryness under reduced pressure to give a syrupy residue. The syrup (0·3 g., 0·0005 mole) was dissolved in anhydrous ether (50 ml.) and a solution of anhydrous hydrogen chloride in ether was added. The *hydrochloride* of the diester (XVI) first appeared as an oil which slowly crystallised; the product was triturated with ether several times before being recrystallised from methanol–ether (×2) (0·142 g., 59%), m. p. 150—151°, $[\alpha]_D^{27}$ +31·0° (*c* 3 in methanol) (Found: C, 66·3; H, 5·8; N, 3·15. $C_{26}H_{25}NO_5$,HCl requires C, 66·7; H, 5·6; N, 2·9%).

N-o-*Nitrophenylsulphenyl*-L-*glutamic Acid* α-*Diphenylmethyl* γ-*Phenacyl Ester* (XI).—The dicyclohexylammonium salt of compound (II) (3·58 g., 0·0055 mole) was converted to the free acid with dilute sulphuric acid as reported above. The resulting oil was dissolved in absolute ethyl acetate (20 ml.) and cooled to 0° before the addition of triethylamine (0·7 ml.) and phenacyl bromide (1·0 g., 0·005 mole). After 10 min. in the cold, the solution was brought to room temperature and set aside 20 hr. It was then washed with water, dilute sulphuric acid, water, and then dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The crystalline product thus obtained was triturated with cold methanol and filtered off to give the pure *diester* (XI) (2·2 g., 75%), m. p. 133—133·5° (unchanged from isopropyl alcohol), $[\alpha]_D^{27}$ −33·8° (*c* 3 in dimethylformamide) (Found: C, 65·9; H, 4·8; N, 4·9. $C_{32}H_{28}N_2O_7S$ requires C, 65·7; H, 4·8; N, 4·8%).

L-*Glutamic Acid* α-*Diphenylmethyl* γ-*Phenacyl Ester* (XII).—This was prepared by dissolution of the corresponding *N*-*o*-nitrophenylsulphenyl derivative (XI) (0·585 g., 0·001 mole) in ethyl acetate (8 ml.) and the addition of hydrogen chloride (3 mol.) in ether. After a few seconds at room temperature, ether was added, to precipitate the *hydrochloride* of (XII) which was filtered off and washed with ether (0·350 g., 75%), m. p. 137° (unchanged from methanol–ethyl acetate), $[\alpha]_D^{27}$ −27° (*c* 3 in methanol) (Found: C, 67·0; H, 5·9; N, 3·2. $C_{26}H_{25}NO_5$,HCl requires C, 66·7; H, 5·5; N, 3·0%).

L-*Glutamic Acid* γ-*Phenacyl Ester Hydrochloride* (XIII).—To a solution of compound (XI) (2·04 g., 0·0035 mole) in nitromethane (31·5 ml.), a solution of hydrogen chloride in nitromethane (2N; 10·5 ml.) was added. A precipitate of the γ-ester hydrochloride began to appear after 5 min. and after 1 hr. at room temperature this precipitate was collected (0·8 g.). A further 5 ml. of hydrogen chloride in nitromethane (2N) were added to the filtrate which was left for a further hour at room temperature after which further precipitate (0·2 g.) was collected. Total yield of the *hydrochloride* (XIII) (1·0 g., 95%), m. p. 166° (from methanol–ethyl acetate), $[\alpha]_D^{20}$ +18° (*c* 3 in methanol) (Found: C, 51·5; H, 5·2; N, 4·6. $C_{13}H_{15}NO_5$,HCl requires C, 51·7; H, 5·3; N, 4·6%).

N-o-*Nitrophenylsulphenyl*-L-*glutamic Acid* γ-*Phenacyl Ester*.—This was prepared by the action of *o*-nitrophenylsulphenyl chloride (0·22 g., 0·001 mole) on compound

(XIII) (0·301 g., 0·001 mole) by procedure *B* already reported.[12] The N-protected γ-ester of L-glutamic acid was isolated as its *dicyclohexylammonium salt* (0·22 g., 37%), m. p. 180—181° (from ethanol), $[\alpha]_D^{27}$ −17·9° (*c* 1 in dimethylformamide) (Found: C, 61·8; H, 6·7; N, 7·2. $C_{31}H_{41}N_3O_7S$ requires C, 62·05; H, 6·9; N, 7·2%).

Glycine β-Phenethyl Ester Hydrochloride.—Dry hydrogen chloride was bubbled through a suspension of glycine (2·8 g., 0·04 mole) in β-phenethyl alcohol (30 ml.) until saturation occurred. The mixture was shaken for 10 hr. at room temperature and then heated under reflux on a water-bath for 1 hr. Upon addition of ether, a mixture of glycine hydrochloride and its ester separated. The free ester was obtained by addition of solid potassium carbonate to a concentrated aqueous solution of the crude product and extraction with ether. The ethereal solution was dried (K_2CO_3) and upon addition of ethereal hydrogen chloride yielded the *hydrochloride* (4·2 g., 50%), m. p. 140—141° (from methanol–ether) (recovery 80%) (Found: C, 55·5; H, 6·7; Cl, 16·55; N, 6·4. $C_{10}H_{13}NO_2$,HCl requires C, 55·7; H, 6·5; Cl, 16·5; N, 6·5%).

The addition of hydrogen bromide to an ethereal solution of the free ester prepared as described above gave the corresponding glycine β-phenethyl ester hydrobromide, m. p. 130° (Found: C, 46·0; H, 5·4; Br, 30·4; N, 5·2. $C_{10}H_{13}NO_2$,HBr requires C, 46·2; H, 5·4; Br, 30·7; N, 5·4%).

Neither the ester hydrochloride nor the hydrobromide in aqueous or alcoholic solution consumed hydrogen in the presence of a palladium catalyst and the starting material could be recovered almost quantitatively.

Hydrogenation of Phenacyl Esters.—(*a*) A solution of glycine phenacyl ester hydrobromide (1·4 g., 0·005 mole) in aqueous methanol was hydrogenated over a catalyst of palladium black, yielding glycine (0·27 g., 72%) as already reported.[4] In the mother-liquor after the isolation of glycine, the presence of glycine β-phenethyl ester could be detected chromatographically.

(*b*) Hydrogenolysis of L-alanine phenacyl ester and of *N*-benzyloxycarbonyl-L-alanyl-glycine phenacyl ester[4] (0·005 mole) in methanol containing small amounts of acetic acid yielded respectively L-alanine and L-alanyl-glycine both in a yield of 70%. L-alanine $[\alpha]_D^{15}$ +14·4° (*c* 2 in 5N-hydrochloric acid) {lit.,[13b] $[\alpha]_D^{25}$ +14·6° (*c* 2 in 5N hydrochloric acid)}; L-alanyl-glycine $[\alpha]_D^{15}$ +51·5° (*c* 2·5 in water) {lit.,[14] $[\alpha]_D^{23}$ +51·3° (*c* 2·5 in water)}.

This investigation was supported by the Royal Hellenic Research Foundation, to which we are greatly indebted.

[7/291 *Received, March 10th*, 1967]

[14] L. Zervas and D. M. Theodoropoulos, *J. Amer. Chem. Soc.*, 1956, **78**, 1359.