N^{ω}-Linked Arginine Peptides [1]

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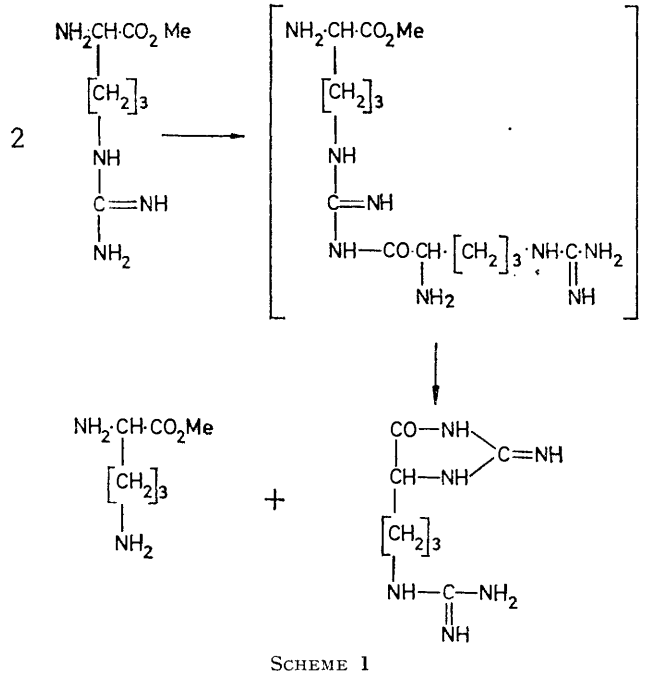
From N^{α}-trityl-L-arginine benzyl ester, N^{ω}-glycyl-, N^{ω}-L-valyl-, and N^{ω}-L-arginyl-L-arginine have been prepared. These non-typical arginine peptides were transformed, especially in alkaline solution, into ornithine and the corresponding 2-iminoimidazolidin-4-one (glycocyamidine) derivatives. In this way the suggestion of Zervas and Bergmann that a non-typical N^{ω}-L-arginyl-L-arginine is formed as an intermediate in the disproportionation of arginine methyl ester was confirmed. When arginine derivatives are used in peptide synthesis, the formation of non-typical arginine peptides can occur. This could lead to the final incorporation of ornithine instead of arginine into the peptide chain.

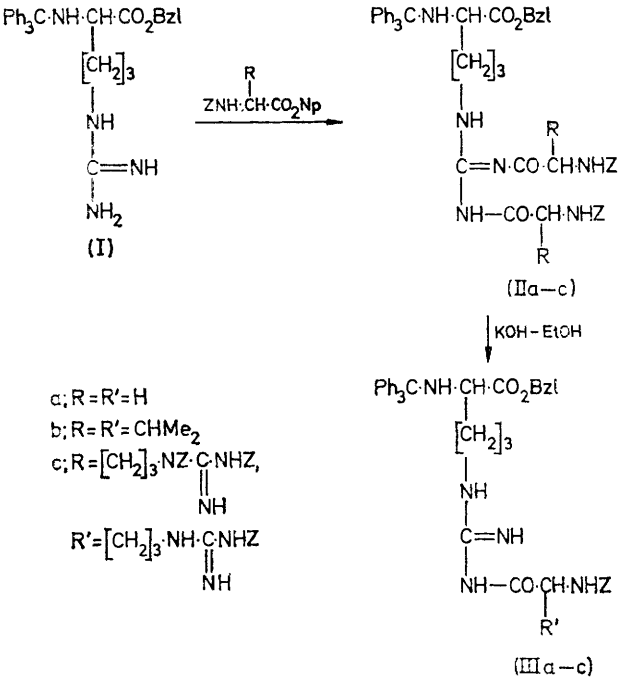
NON-TYPICAL N^{ω}-linked arginine peptides are not encountered in natural products nor, to our knowledge, have they yet been prepared. We report here the preparation of N^{ω}-glycyl-, N^{ω}-L-valyl, and N^{ω}-L-arginyl-L-arginine.†

As starting material N^{α}-trityl-L-arginine benzyl ester [2] (I) was used in order to have the α-NH_2 group temporarily protected and at the same time to avoid an intra- or inter-molecular autocondensation of a free arginine ester *via* the free strongly basic guanidino-group. Indeed,



SCHEME 1

according to Zervas and Bergmann,[3] free arginine ester disproportionates quantitatively to ornithine and a 2-iminoimidazolidin-4-one derivative even at room temperature. These authors considered an N^{ω}-arginyl-arginine as an intermediate in this reaction (Scheme 1).



SCHEME 2

On the other hand the N^{α}-trityl group is known to exert strong steric hindrance in reactions involving the carboxy-group.[4]

The p-nitrophenyl esters of *N*-benzyloxycarbonyl-glycine,[5] *N*-benzyloxycarbonyl-L-valine,[6] and $N^{\alpha},N^{\omega},N^{\omega''}$-trisbenzyloxycarbonyl-L-arginine [7] were coupled with the ester (I) to give arginine derivatives diacylated in the guanidine group ‡ (IIa—c) (Scheme 2). When

† The arginine nitrogen atoms are designated here as follows: $H_2N^{\omega}\text{-}C(:N^{\omega'}H)\text{-}N^{\omega'}H\text{-}[CH_2]_3\text{-}CH(N^{\alpha}H_2)\text{-}CO_2H$.

‡ Bis-(*N*-benzyloxycarbonylaminoacyl)guanidines [8] have been prepared from guanidine and the *p*-nitrophenyl esters of benzyloxycarbonylamino-acids or their mixed anhydrides with ethyl hydrogen carbonate.

[1] I. Photaki, C. Sakarellos, A. Yiotakis, and L. Zervas, 'Peptides: Proceedings of the Thirteenth European Symposium,' ed. Y. Wolman, Wiley–Israel Universities Press, New York–Jerusalem, 1975, p. 65.

[2] C. Sakarellos, Doctoral Dissertation, Division of Natural Sciences (Chemistry Section), University of Athens, 1970.

[3] L. Zervas and M. Bergmann, *Ber.*, 1928, **61**, 1195.

[4] L. Zervas and D. M. Theodoropoulos, *J. Amer. Chem. Soc.*, 1956, **78**, 1359; G. C. Stelakatos, D. M. Theodoropoulos, and L. Zervas, *ibid.*, 1959, **81**, 2884.

[5] M. Bodanszky, *Nature*, 1955, **175**, 685; *Acta Chem. Acad. Sci. Hung.*, 1957, **10**, 335.

[6] B. Iselin, W. Rittel, P. Sieber, and R. Schwyzer, *Helv. Chim. Acta*, 1957, **40**, 373.

[7] E. D. Nicolaides, H. A. Dewald, P. G. Shorley, and H. O. J. Collier, *Nature*, 1960, **187**, 773.

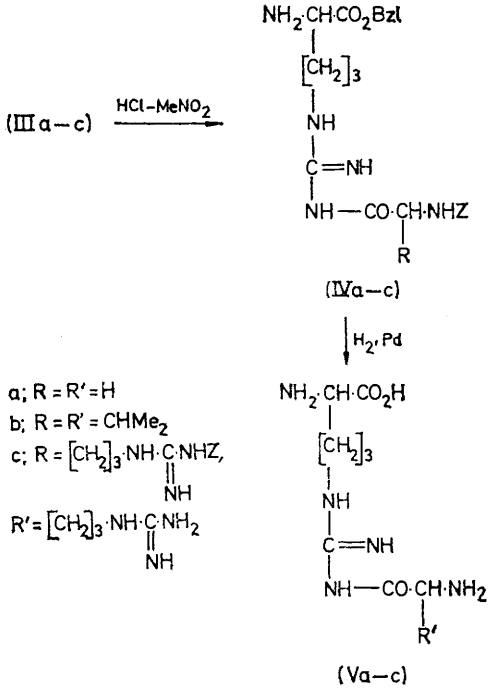
[8] K. Nowak and R. Krug, *Roczniki Chem.*, 1966, **40**, 931; R. Krug and K. Nowak, *ibid.*, 1967, **41**, 1087; 1968, **42**, 333.

N-benzyloxycarbonylglycine succinimido-ester [9] was coupled with arginine benzyl ester (I), the monoacyl compound (IIIa) was isolated as the main product.

The assignment of the $N^{\omega},N^{\omega'}$-structure to the diacyl compounds (IIa—c) was not proved beyond doubt, since we were mainly interested in the monoacyl derivatives (IIIa—c). The presence of one more basic group on compounds (II) was shown by coupling (IIa) with *N*-benzyloxycarbonyl-L-phenylalanine to give the peracylated arginine derivative N^{α}-trityl-$N^{\omega''}$-(benzyloxycarbonyl-L-phenylalanyl)-$N^{\omega},N^{\omega'}$-bis(benzyloxycarbonylglycyl)-L-arginine benzyl ester.

Predictably [10] one acyl group was easily cleaved from compounds (IIa—c) by alkali in the cold, resulting in the monoacyl derivatives (IIIa—c).

From compound (IIIa) the non-typical glycylarginine peptide (Va) was isolated by removing successively the trityl group by the action of anhydrous hydrogen chloride in nitromethane [2] and both the *N*-benzyloxycarbonyl and the benzyl ester group by catalytic hydrogenolysis (Scheme 3).

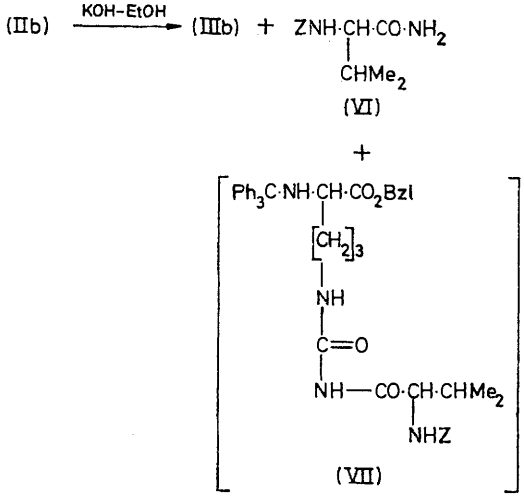


SCHEME 3

The corresponding N^{ω}-valylarginine peptide derivatives were prepared by methods similar to those for the glycylarginine peptides. During the recrystallization of compound (IIIb), *ca.*6% of *N*-benzyloxycarbonyl-L-valinamide (VI) was isolated. The formation of compound (VI) may be explained by a side-reaction (Scheme 4); it also provides evidence for the $N^{\omega},N^{\omega'}$-substitution in compound (IIb). No attempt was made to isolate the citrulline derivative (VII), probably formed simultaneously.

[9] G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Amer. Chem. Soc.*, 1963, **85**, 3039.

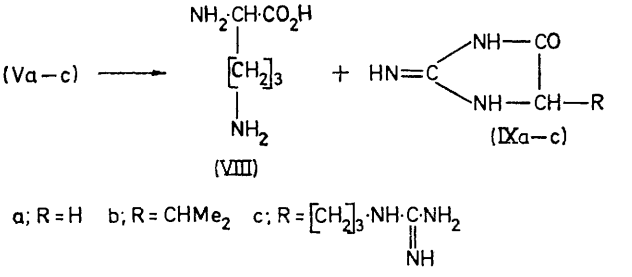
Unlike typical arginine peptides, the non-typical dipeptides (V) are not stable but are easily degraded,



SCHEME 4

being guanidides of α-amino acids,[11] especially at alkaline pH, the products being L-ornithine (VIII) and a heterocyclic compound (IX) (Scheme 5). Thus, N^{ω}-glycyl-L-arginine (Va) affords L-ornithine and 2-iminoimidazolidin-4-one (IXa). This transformation in alkaline solution was followed polarimetrically and shown to be complete in 2 min. The solution was subsequently acidified to pH 6—6.5 and chromatographed on an alumina column. Compound (IXa) was separated from ornithine by elution with water; the former was isolated as its picrate [12] and the latter as its N^{α},N^{δ}-dibenzoyl [3] derivative.

The catalytic hydrogenolysis product of compound (IVb), presumably N^{ω}-L-valyl-L-arginine (Vb), was treated similarly at pH *ca.* 9—10. L-Ornithine was



SCHEME 5

isolated, as its N^{α},N^{δ}-dibenzoyl derivative, but it proved impossible to isolate the 5-isopropylimidazolinone (IXb),

[10] (*a*) L. Zervas, M. Winitz, and J. P. Greenstein, *J. Org. Chem.*, 1957, **22**, 1515; (*b*) L. Zervas, T. Otani, M. Winitz, and J. P. Greenstein, *J. Amer. Chem. Soc.*, 1959, **81**, 2878; (*c*) L. Zervas, M. Winitz, and J. P. Greenstein, *ibid.*, 1961, **83**, 3300.

[11] W. Traube and R. Ascher, *Ber.*, 1913, **46**, 2077, 2083.

[12] H. Tuppy, *Monatsh.*, 1953, **84**, 342.

although the presence of another product was evident from column chromatography on alumina. The expected product (IXb) was synthesised according to a general method.[13]

Finally, treatment of the non-typical dipeptide derivative (IIc) [prepared in the same way (Scheme 2)] with alcoholic potassium hydroxide * followed by removal of the *N*- and *O*-protecting groups gave, presumably, N^{ω}-L-arginyl-L-arginine (Vc) (Scheme 3). This was transformed as expected into the 5-(3-guanidinopropyl)imidazolinone (IXc) and L-ornithine. The former was isolated as its dipicrate, which was converted into the dinitrate.[3] It was identified by colour reactions and by elemental analysis and its melting point was that reported [3] for both L- and the DL-isomers. The optical rotation, however was, as expected, almost zero, indicating autoracemization.[3]

In our opinion, the isolation of ornithine and compound (IXc) is a proof that the intermediates (IIIc—Vc) although not isolated in analytically pure condition, are involved in the foregoing reaction. Furthermore, these findings support the suggestion [3] of the formation of a non-typical L-arginyl-L-arginine peptide as an intermediate in the disproportionation of arginine methyl ester (Scheme 1).

The foregoing results indicate that when arginine derivatives with a free amino-group on the guanidine unit are used in peptide synthesis, non-typical arginine peptides, in addition to the typical ones, can be formed. This could lead to the final incorporation of ornithine instead of arginine into the peptide chain.

EXPERIMENTAL

M.p.s were taken for samples in capillary tubes. Anhydrous solvents were used for removal of the *N*-protecting groups. When necessary, solutions in organic solvents were dried over sodium sulphate. Hydrogenolyses were performed at room temperature and under atmospheric pressure with palladium–charcoal (10%) as catalyst. Before analysis compounds were dried over P_2O_5 at room temperature under high vacuum. Optical rotations were measured with a Perkin-Elmer automatic polarimeter (1 dm cell).

R_F Values refer to t.l.c. on Kieselgel G (Fluka) containing 13% calcium sulphate in the following solvent systems (proportions by volume): (1) butan-1-ol–acetic acid–water (100 : 10 : 30), (2) chloroform–carbon tetrachloride–methanol (6 : 3 : 1), (3) toluene–pyridine–acetic acid (40 : 5 : 0.5), (4) butan-1-ol–acetic acid–water–pyridine (30 : 6 : 24 : 20), (5) acetonitrile–water (3 : 1), (6) cyclohexane–ethyl acetate–methanol (1 : 1 : 1). Thin-layer plates were developed with ninhydrin solution [0.5% in acetone–acetic acid–water (90 : 5 : 5)] or with iodine.

$N^{\omega},N^{\omega'}$-*Bis(benzyloxycarbonylglycyl)*-N^{α}-*trityl*-L-*arginine Benzyl Ester* (IIa).—A solution of N^{α}-trityl-L-arginine benzyl ester toluene-*p*-sulphonate (I) [2] (6.79 g, 0.01 mol) in chloroform (30 ml) was shaken for 3—5 min with sodium hydroxide (2N; 20 ml) in a separatory funnel. The organic layer was washed with water (three times), dried, and cooled. Benzyloxycarbonylglycine *p*-nitrophenyl ester [5] (7.2 g, 0.022 mol) was added and the solution was stirred for 1 h at 0 °C. Next day triethylamine (5 ml) and a few drops of water were added, and the solution was vigorously stirred for 2 h before being washed with water, 5% sodium carbonate solution, and water, then dried and evaporated. The crude product obtained as an oil from ethyl acetate–light petroleum could be crystallized from a solution in methanol by adding water with cooling. Recrystallization by addition of water, with cooling, to a solution in acetonitrile gave the *trisubstituted arginine ester* (5 g, 56%), m.p. 65—70°, $[\alpha]_D^{29}$ +28.2° (*c* 2 in $CHCl_3$), $R_F(1)$ 0.95, $R_F(2)$ 0.95, $R_F(3)$ 0.5 (Found: C, 70.1; H, 6.0; N, 9.6. $C_{52}H_{52}N_6O_8$ requires C, 70.3, H, 5.9; N, 9.5%).

$N^{\omega},N^{\omega'}$-*Bis(benzyloxycarbonylglycyl)*-$N^{\omega''}$-*(benzyloxycarbonyl*-L-*phenylalanyl)*-N^{α}-*trityl*-L-*arginine Benzyl Ester*. To a cold (0 °C) solution of compound (IIa) (0.88 g, 0.001 mol) in dry chloroform (5 ml), *N*-benzyloxycarbonyl-L-phenylalanine (0.32 g, 0.0011 mol) and dicyclohexylcarbodi-imide (0.22 g) were added. After stirring for 1 h at 0 °C the mixture was left at room temperature overnight. Dicyclohexylurea was filtered off and the filtrate washed three times with sodium hydrogen carbonate, then with water, dried, and evaporated. The residue was dissolved in methanol and separated out on addition of water with cooling. The *tetrasubstituted arginine ester* obtained (1 g, 85%) had m.p. 58—60° (decomp.) (unchanged after recrystallization from acetonitrile–water), $[\alpha]_D^{29}$ +29.2° (*c* 2 in $CHCl_3$), $R_F(1)$ 0.95, $R_F(2)$ 0.9, $R_F(3)$ 0.5 (Found: C, 70.3, H, 6.1; N, 8.5. $C_{69}H_{67}N_7O_{11}$ requires C, 70.8; H, 5.8; N, 8.4%).

N^{ω}-*Benzyloxycarbonylglycyl*-N^{α}-*trityl*-L-*arginine Benzyl Ester* (*IIIa*).—(*a*) $N^{\omega},N^{\omega'}$-Bis(benzyloxycarbonylglycyl)-N^{α}-trityl-L-arginine benzyl ester (IIa) (6.3 g, 0.007 mol) was dissolved in alcoholic 0.2N-potassium hydroxide (38 ml) and left at room temperature for 30 min. Upon addition of aqueous sodium chloride solution the *product* separated out as an oil which crystallized after removal of the supernatant liquid, addition of water, and cooling; it was recrystallized from acetonitrile by addition of dilute aqueous sodium chloride; yield 4.4 g (90%); m.p. unsharp.; $[\alpha]_D^{31}$ +33.1° (*c* 2 in $CHCl_3$); $R_F(1)$ 0.9, $R_F(2)$ 0.58, $R_F(3)$ 0.5 (Found: C, 72.0; H, 6.0; N, 9.9. $C_{42}H_{43}N_5O_5$ requires C, 72.3; H, 6.2; N, 10.0%).

(*b*) N^{α}-Trityl-L-arginine benzyl ester toluene-*p*-sulphonate (I) (1.36 g, 0.002 mol) was converted into the free ester as already described. To the cold (0 °C) solution in chloroform was added *N*-benzyloxycarbonylglycine succinimidoester [14] (0.7 g, 0.0022 mol) and the mixture was stirred for 1 h at 0 °C then left at room temperature overnight. Triethylamine (5 ml) and a few drops of water were then added, and the solution was stirred for 2 h, washed several times with water, dried, and evaporated. The residue was dissolved in methanol and the crude product separated out as an oil on addition of water; it was crystallized by decanting the supernatant liquid and adding water again. Finally, it was recrystallized as described under (*a*); yield

* This resulted in cleavage from the ω′-amino-group of compound (IIc) of one trisbenzyloxycarbonyl-L-arginine unit, which at the same time was deprived of its own $N^{\omega''}$-benzyloxycarbonyl group, so that N^{α},N^{ω}-bisbenzyloxycarbonyl-L-arginine [10b] was isolated in 50% yield.

[13] *Cf.* Beilstein: Hauptwerk 1936, vol. 24, p. 244; Zweites Ergänzugswerk, 1954, vol. 24, p. 127.

[14] E. Wünsch, G. Wendlberger, and A. Högel, *Chem. Ber.*, 1971, **104**, 2430.

0.76 g (55%). The product (IIIa) was identified by t.l.c. in systems (1) and (2); $[\alpha]_D^{31}$ +32.8° (*c* 2 in $CHCl_3$).

N$^{\omega}$-Benzyloxycarbonylglycyl-L-arginine Benzyl Ester (IVa). —(a) *Hydrochloride.* To a solution of *N$^{\omega}$*-benzyloxycarbonylglycyl-*N$^{\alpha}$*-trityl-L-arginine benzyl ester (IIIa) (2.8 g, 0.004 mol) in nitromethane (40 ml) 1.5N-hydrogen chloride in nitromethane (9 ml) was added. After 15 min at room temperature the *hydrochloride* was precipitated with ether, filtered off, washed with ether, and dried *in vacuo* (P_2O_5 and NaOH); yield 1.9 g (86%); m.p. unsharp. (Found: C, 48.4; H, 6.4; Cl, 15.7; N, 12.6. $C_{23}H_{29}N_5O_5$,2.5HCl,* H_2O † requires C, 48.9; H, 5.9; Cl, 15.7; N, 12.4%).

(b) *Free base.* A solution of the hydrochloride (1.1 g, 0.002 mol) in water (15 ml) was made alkaline with 10% sodium carbonate. The precipitate was extracted with ethyl acetate (3 times) and the extract washed with water, dried, and evaporated to a small volume. Ether was added to slight turbidity and the mixture was left overnight at 4 °C, whereupon the *free base* crystallized. It was recrystallized from ethyl acetate; yield 0.68 g (75%); m.p. 101—103°; $[\alpha]_D^{28}$ −10.8° (*c* 2 in $CHCl_3$); $R_F(1)$ 0.5, $R_F(4)$ 0.86 (Found: C, 60.4; H, 6.4; N, 15.2. $C_{23}H_{29}N_5O_5$ requires C, 60.65; H, 6.6; N, 15.4%).

N$^{\omega}$-Glycyl-L-arginine Bishydrochloride (Va).—Compound (IVa) (1.06 g, 0.002 mol) in 95% methanol (25 ml) was hydrogenated over palladium–charcoal (0.2 g). The mixture was filtered through Celite and the filter pad washed with 95% methanol. The filtrate was evaporated to dryness and the residue was crystallized by addition of ethyl alcohol with cooling. The *dipeptide salt* was recrystallized from water–acetone; yield 0.4 g (66%); m.p. 133° (decomp.); $[\alpha]_D^{31}$ +11.2° (*c* 2 in H_2O) (Found: Cl, 20.6; N, 20.5. $C_8H_{17}N_5O_3$,2HCl,2H_2O requires Cl, 20.8; N, 20.6%).

N$^{\omega}$,N$^{\omega'}$-Bis(benzyloxycarbonylglycyl)-L-arginine Benzyl Ester Hydrochloride.—Compound (IIa) (0.45 g, 0.0005 mol) was dissolved in a solution of hydrogen chloride in nitromethane (0.34N; 5 ml). After 15 min at room temperature ether was added and the *hydrochloride* separated; recrystallized from acetone–ether (yield 0.31 g, 87%) it had m.p. 80—90°, $[\alpha]_D^{19.5}$ −7.5° (*c* 3 in $CHCl_3$), $R_F(1)$ 0.8, $R_F(2)$ 0.4 (Found: C, 55.2; H, 5.8; Cl, 7.3; N, 11.8. $C_{33}H_{38}N_6O_8$,-1.5HCl,H_2O requires C, 55.1; H, 5.8; Cl, 7.4; N, 11.7%).

Transformation of N$^{\omega}$-Glycyl-L-arginine Peptides and Isolation of 2-Iminoimidazolidin-4-one (IVa) *and L-Ornithine* (VIII).—(*a*) To a solution of compound (Va) (0.17 g, 0.0005 mol) in water, ammonium hydroxide (2N; 1 ml) was added (to pH 9—10). After 5 min the solution was acidified with N-hydrochloric acid (pH 6—6.5) and chromatographed on a column (2 × 18 cm) of aluminium oxide (Fluka type 506 C, weakly acidic, activity I). Compounds were eluted with water (2 ml fractions). In a typical experiment the imidazolidinone was eluted in fractions 5—10 [detected on thin-layer plates with iodine; solvent system (5)] and ornithine in fractions 11—36 [detected on t.l.c. plates with ninhydrin; solvent systems (5) and (7)]. From the imidazolidinone-containing fractions after evaporation to a small volume and addition of picric acid (0.11 g, 0.0005 mol), the crystalline picrate salt separated (0.098 g, 60%), m.p. and mixed m.p. 209—210° (lit.,[11] 209—210°). The imidazolidinone was also identified by characteristic colour reactions.[13] From the ornithine fractions after evaporation and benzoylation, dibenzoyl-L-ornithine (0.05 g, 30%) was obtained, m.p. 183—184° (after recrystallization from 40% ethyl alcohol and addition of 1—2 drops of concentrated hydrochloric acid), $[\alpha]_D^{31}$ +9.9° (10% in water containing 1 equiv. of potassium hydroxide) {lit.,[3] m.p. 187°, $[\alpha]_D$ +8.8° (10% in water containing 1 equiv. of potassium hydroxide)} (Found: C, 67.0; H, 5.7; N, 8.0. Calc. for $C_{19}H_{20}N_2O_4$: C, 67.0; H, 5.9; N, 8.2%).

(b) *N$^{\omega}$,N$^{\omega'}$*-Bis(benzyloxycarbonylglycyl)-L-arginine benzyl ester hydrochloride (2.16 g, 0.003 mol) was hydrogenated in 95% methanol (25 ml) as described for the preparation of substance (Va). The product, presumably *N$^{\omega}$,N$^{\omega'}$*-diglycyl-L-arginine hydrochloride, was similarly isolated (0.6 g), m.p. 202—204°. This substance (0.18 g) was treated for 30 min with ammonium hydroxide; the product was acidified and chromatographed as described under (*a*) to yield, after recrystallization, the imidazolidinone picrate (0.1 g), m.p. 207—209°, dibenzoyl-L-ornithine (0.11 g), m.p. 182—183°, $[\alpha]_D^{31}$ +9.5° (10% in water containing 1 equiv. of potassium hydroxide).

Time-dependence of L-Ornithine Appearance during Treatment of N$^{\omega}$-Glycyl-L-arginine with Ammonium Hydroxide.—A solution of compound (Va) (0.02 g, 0.00069 mol) in water (2 ml) shows α_{589} 0.112° (*l* 1 dm, 31 °C). After addition (at *t* = 0) of 5N-ammonium hydroxide (final volume 2.2 ml) the angle of rotation was changed as follows:

t/min	1	1.5	2
α/°	0.085	0.067	0.054 (final value)

On the assumption that the final value is due to L-ornithine liberated quantitatively from compound (Va), the calculated specific optical rotation for L-ornithine is +12.9°. L-Ornithine monohydrochloride, treated exactly as above, showed a specific optical rotation of +12.5°.

N$^{\omega}$,N$^{\omega'}$-Bis(benzyloxycarbonyl-L-valyl)-N$^{\alpha}$-trityl-L-arginine Benzyl Ester (IIb).—*N*-Benzyloxycarbonyl-L-valine *p*-nitrophenyl ester [6] (1.48 g, 0.004 mol) was coupled with *N$^{\alpha}$*-trityl-L-arginine benzyl ester (0.002 mol) by a procedure similar to that used for compound (IIa). After evaporation of chloroform the residue was dissolved in methanol. Addition of water precipitated *compound* (IIb) as an oil which was solidified by decanting the supernatant liquid and adding water. It was recrystallized from acetonitrile–water with cooling; yield 1.1 g (55%); m.p. 66—69° (decomp., after softening at 57°); $[\alpha]_D^{28}$ +8.2° (*c* 2 in $CHCl_3$), $R_F(1)$ 0.95, $R_F(2)$ 0.95, $R_F(3)$ 0.55 (Found: C, 71.4; H, 6.6; N, 8.45. $C_{58}H_{64}N_6O_8$ requires C, 71.5; H, 6.6; N, 8.6%).

N$^{\omega}$-Benzyloxycarbonyl-N$^{\alpha}$-trityl-L-valyl-L-arginine Benzyl Ester (IIIb).—Compound (IIb) (6.3 g, 0.000647 mol) was treated with alcoholic potassium hydroxide as described for compound (IIa) to yield the crude product (5.2 g, 90%), m.p. 62—70° (decomp.). For recrystallization this material (0.7 g) was dissolved in acetonitrile (3 ml). Some crystalline benzyloxycarbonyl-L-valinamide (VI) was filtered off {0.044 g; m.p. and mixed m.p.‡ 197—199°; $[\alpha]_D^{19.5}$ +24.6° (*c* 1.5 in Me_2N·CHO) (Found: C, 62.4; H, 7.25; N, 11.0.

* Many crystalline salts of arginine derivatives prepared in this laboratory contain acid in non-stoicheiometric quantity.[2]

† In all compounds in this paper, in addition to elemental analysis, the water content was determined by the Karl Fisher method.

‡Authentic material synthesized by bubbling ammonia through a cooled solution of *N*-benzyloxycarbonyl-L-valine methyl ester in dry methanol; m.p. 201—203° (from acetonitrile), $[\alpha]_D^{19.5}$ +24.2° (*c* 1 in Me_2N·CHO) [lit.,[14] m.p. 212°; $[\alpha]_D^{20}$ +22.6° (*c* 1 in Me_2N·CHO)].

$C_{13}H_{18}N_2O_3$ requires C, 62.4; H, 7.25; N, 11.2%)}. From the filtrate, *compound* (IIIb) was precipitated by addition of water; (yield 0.630 g, 90%), m.p. 62—70°, $[\alpha]_D^{24}$ +27.7° (*c* 2 in $CHCl_3$) (Found: C, 73.7; H, 6.9; N, 8.9. $C_{45}H_{49}N_5O_5$ requires C, 73.0; H, 6.7; N, 9.5%).

N^{ω}-*Benzyloxycarbonyl*-L-*valyl*-L-*arginine Benzyl Ester* (IVb) *Oxalate*.—Compound (IIIb) (0.74 g, 0.0001 mol) was dissolved in nitromethane (3 ml), 1.25N-hydrogen chloride in nitromethane (2.6 ml) was added, and the solution was worked up as described for compound (IVa) to give the hydrochloride salt (0.4 g), m.p. 90—102°, converted into the oxalate as follows. The hydrochloride (1.14 g) was dissolved in water (15 ml) and the solution was made alkaline with 10% sodium carbonate. The precipitated solid was extracted with ethyl acetate. The ethereal layer was washed twice with water, dried, and evaporated to *ca.* 10 ml. A solution of oxalic acid (0.54 g) in ethyl acetate (10 ml) and methanol (3 ml) was added and the solution was left in an unstoppered flask for 3 h at room temperature, whereupon the crystalline *oxalate* started to separate. It was left overnight at 4 °C, filtered off, and recrystallized from methanol–ethyl acetate; m.p. 152—154°, $[\alpha]_D^{24}$ −15.7° (*c* 2 in MeOH), $R_F(1)$ 0.6, $R_F(2)$ 0.4 [Found: C, 51.7; H, 5.6; N, 10.0. $C_{26}H_{35}N_5O_5,2(CO_2H)_2,1.5\ H_2O$ requires C, 51.1; H, 5.9; N, 9.9%]. The oxalic acid content, determined by titration with potassium permanganate solution, was 25.8% (calc. 25.5%).

Transformation of N^{ω}-L-*Valyl*-L-*arginine Derivatives; Isolation of* L-*Ornithine*.—Compound (IVb) oxalate (1.16 g, 0.00164 mol) was hydrogenated in 95% methanol (catalyst 0.2 g). The catalyst was filtered off through Celite and the filtrate evaporated to dryness. The product, presumably N^{ω}-L-valyl-L-arginine (Vb) oxalate, separated out on the addition of ethyl alcohol (yield 0.4 g; m.p. 98—104°). To a solution of this substance (0.2 g) in water (2 ml), 5N-ammonium hydroxide was added (to pH 9—10). After 15 min the solution was acidified (pH *ca.* 6.5) with N-hydrochloric acid and chromatographed as described for the isolation of the transformation products of compound (Va). A ninhydrin-positive substance was isolated from fractions 21—40 but it was not identified. [It decomposed at 230 °C; an aqueous solution had pH 8; the picric acid (in Na_2CO_3) and Sakaguchi reactions [13] were negative.] The combined eluate from fractions 42—180, after evaporation and benzoylation, gave dibenzoyl-L-ornithine (0.026 g), m.p. 181—182°, $[\alpha]_D^{29}$ +9.5° (10% in water containing 1 equiv. potassium hydroxide).

L-α-*Guanidinoisovaleric Acid*.—To a solution of L-valine (9.44 g, 0.08 mol) in concentrated ammonium hydroxide (160 ml), *S*-methylisothiourea (7.5 g, 0.059 mol) was added with stirring. After 48 h at room temperature, the *product* was filtered off and washed with water; yield 0.4 g, (14%); m.p. 251—252° (decomp.) (from water); $[\alpha]_D^{31}$ −3.1° (*c* 2 in N-HCl) (Found: C, 45.4; H, 8.3; N, 26.5. $C_6H_{13}N_3O_2$ requires C, 45.3; H, 8.2; N, 26.4%).

2-*Imino*-5-*isopropylimidazolidin*-4-*one* (IXb).—A solution of the foregoing compound (0.3 g) in concentrated hydrochloric acid was evaporated on a steam-bath. The process was repeated and the crystalline residue was dissolved in a small quantity of water and precipitated by neutralization with 5N-ammonium hydroxide. The *product* was filtered off, washed with cold water, and dried; yield 0.15 g (58%); m.p. 256—257° (decomp.) (from water) (Found: C, 48.2; H, 8.1; N, 27.6. $C_6H_{11}N_3O,0.5\ H_2O$ requires C, 47.9; H, 8.0; N, 28.0%).

$N^{\omega},N^{\omega'}$-*Bis*($N^{\alpha},N^{\omega},N^{\omega''}$-*trisbenzyloxycarbonyl*-L-*arginyl*)-N^{α}-*trityl*-L-*arginine Benzyl Ester* (IIc).—$N^{\alpha},N^{\omega},N^{\omega''}$-Tris-benzyloxycarbonyl-L-arginine *p*-nitrophenyl ester [7] (1.39 g, 0.002 mol) was coupled with N^{α}-trityl-L-arginine benzyl ester (0.001 mol) by a procedure similar to that used for compound (IIa). The chloroform layer was evaporated to dryness and the residue was crystallized by addition of ethyl alcohol–water with cooling, giving *compound* (IIc) (1.2 g, 75%), m.p. 70—80° (decomp., softening at 60°), unchanged after recrystallization from acetonitrile–cold water, $[\alpha]_D^{28}$ +15.5° (*c* 2 in $CHCl_3$), $R_F(1)$ 0.9; $R_F(2)$ 0.83 (Found: C, 68.1; H, 6.0; N, 10.2. $C_{92}H_{94}N_{12}O_{16}$ requires C, 68.0; H, 5.8; N, 10.35%).

Transformation of N^{ω}-L-*arginyl*-L-*arginine Derivatives; Isolation of* 5-(3-*Guanidinopropyl*)-2-*iminoimidazolidin*-4-*one* (IXc) *and* L-*Ornithine*.—Compound (IIc) (2.43 g, 0.0015 mol) was dissolved in alcoholic potassium hydroxide (0.36N; 18.6 ml) with gentle heating. After 1 h at room temperature water was added and the mixture evaporated to remove ethyl alcohol. The residue was twice extracted with ethyl acetate. The cooled aqueous layer was acidified with acetic acid to give N^{α},N^{ω}-bisbenzyloxycarbonyl-L-arginine (0.3 g, 50%), m.p. 148—150° (lit.,[3b] 148—150°). The organic layer was washed with dilute aqueous sodium hydroxide (twice), with water (three times), then dried and evaporated to small volume. Ethyl alcohol was added and the mixture was evaporated to dryness. Upon addition of water with cooling, the product, presumably compound (IIIc), crystallized [1.2 g; m.p. 85—90° (decomp.)]. It was detritylated by treatment with 0.5N-hydrogen chloride in nitromethane (39 ml for 3.7 g of substance). After 15 min at room temperature, ether was added to precipitate (presumably) N^{ω}-(N^{α},N^{ω}-bisbenzyloxycarbonyl-L-arginyl)-L-arginine benzyl ester (IVc) hydrochloride [2.8 g; m.p. 102—105° (unsharp.)]. Finally the benzyloxycarbonyl and the benzyl ester groups were removed from compound (IVc) by catalytic hydrogenolysis in 85% methanol containing 2% acetic acid. Filtration and evaporation left an oily residue. An aqueous solution of this oily product (1.7 g), presumably N^{ω}-L-arginyl-L-arginine (Vc) hydrochloride, was made strongly alkaline by adding concentrated ammonium hydroxide. After 30 min the excess of ammonia was removed *in vacuo*, picric acid (3 g) was added, and the mixture was refluxed for 1 h on a steam-bath. Next day the supernatant liquor was decanted, water was added and decanted three times, and the 5-(3-guanidinopropyl)imidazolidinone picrate (0.5 g) was crystallized by addition of ethyl alcohol; m.p. 198—200° (decomp.). For purification this picrate was dissolved in 50% ethyl alcohol (40 ml) by gentle heating, nitric acid (1N; 2.85 ml) was added at room temperature, and the solution was repeatedly extracted to remove completely the picric acid. The solution was triturated with active charcoal and evaporated to *ca.* 4 ml. After scratching and cooling, the crystalline *dinitrate* separated out; m.p. 182° (decomp.) [after recrystallization from methanol–ether, m.p. 186—187° (decomp.)]; $[\alpha]_D^{25}$ −0.8° (*c* 2 in H_2O) [lit.,[3] m.p. 189° (corr.)] (Found: C, 26.2; H, 5.1; N, 34.3. $C_7H_{16}N_8O_7$ requires C, 25.9; H, 5.0; N, 34.6%).

From the aqueous mother liquors (from decantation and the picrate filtrates) dibenzoyl-L-ornithine was isolated as follows. The solution was acidified with concentrated hydrochloric acid, whereupon picric acid separated out and was filtered off. The filtrate was extracted with ether until decolourized, then evaporated to dryness, and the residue was benzoylated to yield dibenzoyl-L-ornithine (0.3 g

recryst.), m.p. 182—183°, $[\alpha]_D^{28}$ +9.3° (10% in water containing 1 equiv. potassium hydroxide).

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