126. *Amino-acids and Peptides. Part XXI.*[1] *Removal of the Nitro-group from Nitroarginine and Peptides of Nitroarginine by Electrolytic Reduction.*

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Nitro-L-arginine can be reduced at a mercury cathode to L-arginine nearly quantitatively, and this new method for the removal of the nitro-group has been used in the synthesis of L-arginyl-L-leucine, -L-valine, -glycine, -L-proline, -*S*-benzyl-L-cysteine, and -*S*-benzylthiomethyl-L-cysteine, and of L-prolyl-L-arginine. The procedure succeeds with sulphur-containing peptides, for which catalytic hydrogenation fails. By the use of a mixture of tetrahydrofuran and N-sulphuric acid as solvent during the reduction, N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginylglycine was converted into N^{α}-benzyloxycarbonyl-L-arginylglycine.

Protection of the guanidino-group in arginine during peptide synthesis has been achieved by nitration,[2] dibenzyloxycarbonylation,[3,4] tosylation,[5] and by working at pH values at which this group is protonated.[6] Although the protection afforded by nitration is incomplete, and side-reactions can occur under certain conditions,[7,8] many peptides of arginine have been synthesised satisfactorily in this way, as for example, in the recent work of Gibian and Schröder.[9] However, the removal of the protecting nitro-group has so far been achieved only by catalytic hydrogenation, which usually fails in the presence of sulphur. Attempts to use sodium in liquid ammonia or tin and hydrochloric acid for this purpose were unsuccessful;[10] hydrogenation failed to remove the nitro-group from (N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl)-N^{ω}-benzyloxycarbonyl-L-ornithine and -L-lysine,[11] and with larger peptides the hydrogenation is slow and irreproducible.[12] As briefly reported earlier,[8] we have found that electrolytic reduction in acid solution at a mercury cathode reduces nitro-L-arginine nearly quantitatively to L-arginine, and the same procedure has proved successful for nitroarginyl peptides.

It is important in these reductions that the time required under the conditions used should be determined in each case, and the progress of the reaction is readily followed by means of paper electrophoresis at pH 6 or 11·5. At the latter pH, ornithine can be detected in the presence of arginine, but we have found none nor have we found evidence of the presence, in the products of reduction under our conditions, of the aminoguanidine intermediate referred to by Gros *et al.*[4] If the reduction is unduly prolonged, by-products are formed and the peptide may then be difficult to purify. We investigated the use of low, controlled cathodic potentials, but the time required increased considerably and in the cases examined the product was less readily purified. Since electrolytic reductive fission of the peptide bond of peptides of proline has been reported,[13] we submitted L-arginyl-L-proline to further reduction; after 12 hours a faint ninhydrin-positive impurity was revealed by paper electrophoresis at pH 6, but it was only after 16 hours that evidence of proline appeared, and it is clear that the peptide bond is not reduced under our conditions. An interesting extension of this method is the use of a mixed solvent for the reduction of

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TABLE 1.

Dipeptides of nitroarginine.

No.	Dipeptide	Yield (%)	M. p.	R_F [α]$_D$ [a]	R_F BWA	R_F PW
1	Nitro-L-arginyl-L-leucine	87	233—235° [b]	+11·0	0·65	0·80
2	,, -L-valine	98	207	+20	0·56	0·77
3	,, -glycine	91	163—165 [c]	+50	0·18	0·59
4	,, -L-proline (acetate) [d]	90	133—134	−50	0·26	0·85
5	,, -*S*-benzyl-L-cysteine [e]	85	169—170	−9·2 [f]	0·66	0·86
6	,, -*S*-benzylthiomethyl-L-cysteine [e]	95	174—175	−21·5 [f]	0·74	0·95
7	L-Prolyl-*N*ω-nitro-L-arginine	85	212—214 [g]	−24 [f]	0·38	0·93

No.	Temp. of drying (at 0·05 mm.) for analysis	Found (%) C	H	N	S	Formula	Required (%) C	H	N	S
1	60°	43·4	7·45	24·85	—	$C_{12}H_{24}N_6O_5$	43·4	7·3	25·3	—
2	60	41·6	7·2	26·8	—	$C_{11}H_{22}N_6O_5$	41·5	6·9	26·4	—
3	60	34·8	5·8	29·7	—	$C_8H_{16}N_6O_5$	34·8	5·8	30·4	—
4	60	41·25	6·6	22·3	—	$C_{11}H_{20}N_6O_5,CH_3{\cdot}CO_2H$	41·5	6·4	22·3	—
5	20	46·2	6·1	20·1	7·5	$C_{16}H_{24}N_6O_5S$	46·6	5·9	20·4	7·75
6	60	44·3	5·8	18·1	13·6	$C_{17}H_{26}N_6O_5S_2$	44·5	5·7	18·3	13·95
7	100	41·6	6·7	26·3	—	$C_{11}H_{20}N_6O_5$	41·8	6·4	26·6	—

[a] *c* 1—1·7 in H_2O at 20—23° unless otherwise stated. [b] After previously melting at 189—190° and resolidifying. [c] From methanol. [d] Debenzyloxycarbonylation took 30 min. [e] Debenzyloxycarbonylation carried out in the presence of ethyl methyl sulphide. [f] *c* 0·9—1·1 in 95% acetic acid. [g] From aqueous methanol.

TABLE 2.

Electrolytic reduction of nitroarginine and related peptides.

Wt. of nitro-compound (mg.)	Product	Reduction time (hr.)	Yield (%)	M. p.	[α]$_D$ [a] Obs.	[α]$_D$ [a] Lit.
400	L-Arginine acetate [b]	6·5	90	217—218°	+21° [c]	+18·9 [d]
400	L-Arginyl-L-leucine acetate [e]	3·75	95	183—185	+8·6	+8·9 to +11·6 [f–j]
400	,, -L-valine acetate	5·0	85	203	+12·5 [k]	+12·3,[l] +15·8 [i]
400	,, -glycine diacetate	5·0	80	148—150 [m]	+36	+37·6 to +47·1 [f,h,i,]
200	,, -L-proline acetate [n]	1·0	95		−57	New cpd.
400	,, -*S*-benzyl-L-cysteine acetate [o]	3·25	91	101—103	+2·3 [p] −5·2 [q]	New cpd.
200	,, -*S*-benzylthiomethyl-L-cysteine acetate [r]	1·5	84	132—134	−16·5 [q]	New cpd.
200	L-Prolyl-L-arginine acetate [s]	1·0	85	153—155	−29	−26·9,[l] −28·6 [h]

[a] *c* 0·9—1·8 in H_2O at 20—23° unless otherwise stated. [b] After evaporation of the solution addition of ethanol caused crystallisation. [c] *c* 4·0 in 6N-HCl at 24°. [d] Calculated from the figure quoted in ref. 6. [e] Physical constants refer to material recrystallised from acetic acid–methanol. [f] Berse and Piché, *J. Org. Chem.*, 1956, **21**, 808. [g] Izumiya and Makisumi, *J. Chem. Soc. Japan*, 1957, **78**, 1768. [h] Ref. 15. [i] Zervas, Otani, Winitz, and Greenstein, *J. Amer. Chem. Soc.*, 1959, **81**, 2878. [j] Ref. 6. [k] At 26°. [l] Ref. 9. [m] From acetic acid–ethyl acetate (lit.,[15] m. p. 166—168°). Recrystallisation with seeding gave the higher-melting form; a mixture of the two forms melted at 166—168°. [n] Precipitated from ethanol by ether (Found, on a sample dried at 20°/0·05 mm.: C, 45·0; H, 7·8; N, 19·6. $C_{13}H_{25}N_5O_5,H_2O$ requires C, 44·7; H, 7·8; N, 20·0%). [o] Evaporation left a hygroscopic white powder which became stable after keeping over potassium hydroxide pellets (Found: C, 48·3; H, 7·2; N, 16·2; S, 7·4. $C_{18}H_{29}N_5O_5S,H_2O$ requires C, 48·5; H, 7·0; N, 15·7; S, 7·2%). [p] In MeOH. [q] In 95% acetic acid. [r] Precipitated from methanol by ether and kept over potassium hydroxide pellets (Found, on a sample dried at 20°/0·05 mm.: C, 46·4; H, 6·65; N, 14·5; S, 13·3. $C_{19}H_{31}N_5O_5S_2,H_2O$ requires C, 46·4; H, 6·8; N, 14·2; S, 13·1%). [s] The glass remaining after evaporation of the solution was dissolved in warm ethanol, and addition of ether gave the crystalline monohydrate.

N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginylglycine to N^{α}-benzyloxycarbonyl-L-arginylglycine (in 62% yield after recrystallisation).

For the preparation of N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginylamino-esters we used the carbonic mixed anhydride procedure [14,15] but we found dimethylformamide a more

[14] Hofmann, Rheiner, and Peckham, *J. Amer. Chem. Soc.*, 1953, **75**, 6083.
[15] Hofmann, Peckham, and Rheiner, *J. Amer. Chem. Soc.*, 1956, **78**, 238.

effective solvent than dioxan. In view of the possibility of lactam formation [7,8] the conditions for this reaction must be carefully controlled and therefore we describe our procedure. Hydrolysis of the esters, and then treatment of the acids with hydrogen bromide in acetic acid, readily gave the *nitroarginyl dipeptides* (Table 1) required for the reductions; the results of the reductions are recorded in Table 2. It will be seen that the sulphur-containing peptides L-arginyl-*S*-benzyl-L-cysteine and L-arginyl-*S*-benzylthiomethyl-L-cysteine were prepared in good yield by this route.

Experimental

Melting points were determined on a Kofler hot-stage apparatus. The solvents for paper chromatography were: " BWA ": butan-1-ol–water–acetic acid (62 : 26 : 12 by volume, freshly mixed); " PW ": phenol saturated with water; descending flow, with Whatman No. 4 paper. Electrophoresis was on the same paper, at 17v/cm., using phosphate (pH 6) or sodium carbonate buffer (pH 11·5). Optical rotations were recorded on an Ericson automatic polarimeter. Evaporation was usually by rotary evaporator and solutions in organic solvents were dried over $MgSO_4$.

Nitro-L-arginine was prepared either from L-arginine,[15] or from its hydrochloride.[9] N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginine was prepared as described for the D-isomer.[9] To obtain material of good optical rotation it was important to keep the pH above 10·5 during the reaction; it seems likely that at lower alkalinity some mixed anhydride may be formed, which could give rise to racemic product either directly or through the lactam.[7,8] With this precaution, crude product of $[\alpha]_D^{19}$ −2·8 to −3·0° (*c* 5 in MeOH), $[\alpha]_D^{19}$ −13 to −14° (*c* 2·5 in pyridine) was readily obtained {lit., $[\alpha]_D^{27}$ −3·5° (*c* 1·02 in MeOH); [15] $[\alpha]_D^{23}$ −3·5° (*c* 2 in MeOH),[9] and for the the D-isomer, $[\alpha]_D^{23}$ +2·8° (*c* 2 in MeOH) [9]}.

General Procedure for Coupling N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\omega}$-*nitro*-L-*arginine with Amino-esters.*—The method of Hofmann, Peckham, and Rheiner,[14,15] was modified as follows. N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginine (0·036 mole) was dissolved in dimethylformamide (70 ml.) containing triethylamine (0·036 mole) and the solution was cooled to 0°. Ethyl chloroformate (0·036 mole) was added and the solution was stirred for 4 min.; distilled amino-ester (0·036 mole) (or amino-ester hydrochloride with an equivalent of triethylamine) was added and washed in with dimethylformamide (20 ml.). The mixture was stirred for 3 hr. at 20°, poured into N-hydrochloric acid (400 ml.) previously cooled to −10°, and precipitation was completed during 10 hr. at 0°; in the case of the analogues containing cysteine, some or all of the dimethylformamide was evaporated under reduced pressure before acidification. The white product was collected and washed with N-hydrochloric acid and water. It was dispersed in saturated aqueous sodium hydrogen carbonate, the solid was again collected, washed on the filter with aqueous sodium hydrogen carbonate and water, and dried. The yields and new constants were as follows.

N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-leucine methyl ester (74% after recrystallisation from methanol–ether), $[\alpha]_D^{22}$ −7·0 (*c* 1·2 in dimethylformamide), $[\alpha]_D^{23}$ −15° (*c* 1·0 in pyridine). N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-valine methyl ester (66% after recrystallisation from methanol), $[\alpha]_D^{24}$ −2·3° (*c* 1·0 in dimethylformamide); N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginylglycine ethyl ester (prepared using tri-n-butylamine in place of triethylamine), (57% after recrystallisation from ethyl acetate), m. p. 117—118°, $[\alpha]_D^{20}$ −10·8° (*c* 1·0 in MeOH); material prepared by the dicyclohexylcarbodi-imide method, with tetrahydrofuran–chloroform as solvent,[9] had the same m. p. and $[\alpha]_D^{22}$ −10·3° (*c* 2·1 in MeOH). Many repetitions and changes in conditions gave similar product {lit.,[9] m. p. 77—78° or 118—120°, $[\alpha]_D$ −13·4 to −15·8° (*c* 2 in MeOH) at 27—22°}. Dr. E. Schröder kindly repeated this preparation and allows us to quote amended figures of $[\alpha]_D^{20}$ −11·8 and −11·5 ± 0·5° (*c* 1 in MeOH) for material prepared by the dicyclohexylcarbodi-imide method. Further evidence on the optical purity of this ester and of its methyl analogue will be given in a later Paper of this series. N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-*S*-benzyl-L-cysteine methyl ester (71% after recrystallisation from ethanol), $[\alpha]_D^{19\cdot5}$ −26° (*c* 1·0 in dimethylformamide). N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\omega}$-*nitro*-L-*arginyl*-S-*benzylthiomethyl*-L-*cysteine methyl ester* (82%), m. p. 126·5—127·5° (from methanol), $[\alpha]_D^{20}$ −30° (*c* 1·0 in dimethylformamide) (Found: C, 51·5; H, 5·4; N, 13·3. $C_{26}H_{34}N_6O_7S_2$ requires C, 51·5; H, 5·6; N, 13·9%).

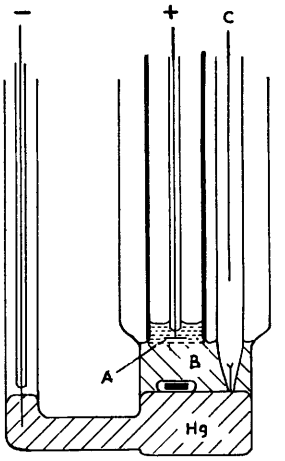
N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\omega}$-*nitroarginyl-amino-acids.*—These were prepared in the normal way

by hydrolysis of the corresponding ester by means of aqueous N-sodium hydroxide in methanol or in tetrahydrofuran at room temperature. The following new constants were recorded.

N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-valine, $[\alpha]_D^{21}$ $-3{\cdot}7°$ (*c* 1·0 in EtOH), $[\alpha]_D^{21}$ $-6{\cdot}1°$ (*c* 1·0 in dimethylformamide) {lit.,[9] $[\alpha]_D^{28}$ $-2{\cdot}9°$ (*c* 2 in EtOH)}. N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginylglycine (85%), $[\alpha]_D^{21}$ $-10{\cdot}2°$ (*c* 1·0 in MeOH) {lit., $[\alpha]_D^{23}$ $-15{\cdot}2°$ (*c* 1 in MeOH),[9] $[\alpha]_D^{28}$ $-16{\cdot}8°$ (*c* 1 in MeOH) [15]}. Dr. E. Schröder has kindly checked this preparation and allows us to quote an amended figure of $[\alpha]_D^{23}$ $-10{\cdot}2°$ (*c* 1 in MeOH), unchanged on recrystallisation. Further evidence on the optical purity of this compound will be given in a later Paper of this series. N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-*S*-benzyl-L-cysteine, $[\alpha]_D^{23}$ $-16°$ (*c* 1·0 in dimethylformamide). N^{α}-*Benzyloxycarbonyl*-N^{ω}-*nitro*-L-*arginyl*-S-*benzylthiomethyl*-L-*cysteine*, prepared in 85% yield by hydrolysis with 2·4 equivalents of N-sodium hydroxide in tetrahydrofuran for 24 hr., had m. p. 149—150°, $[\alpha]_D^{22{\cdot}5}$ $-34°$ (*c* 0·9 in MeOH) (Found: C, 51·1; H, 5·6; N, 14·7. $C_{25}H_{32}N_6O_7S_2$ requires C, 50·7; H, 5·4; N, 14·2%).

N^{ω}-*Nitro*-L-*arginyl-amino-acids*.—The N^{α}-benzyloxycarbonyl derivatives (*ca.* 0·004 mole) were dissolved in *ca.* 2·1N-hydrogen bromide in acetic acid (to provide 2·5 mol. of HBr); after

Apparatus for electrolytic reduction.



1½ hr. (except when otherwise stated in Table 1) at room temperature the solution was poured into ether; the dipeptide hydrobromide was extracted into water, and the aqueous solution was passed through a column of Dowex-3 acetate resin; where necessary, elution was completed by dilute acetic acid. The eluate, which was free from bromide, was evaporated at 30°/0·05 mm. leaving a glass; in most cases, this glass dissolved in a small volume of warm absolute methanol but soon the solution deposited the dipeptide as a white solid, insoluble in methanol, and chromatographically, electrophoretically and analytically pure; where necessary, ether was added to precipitate the product from the methanol. In the case of cysteine derivatives the reaction was carried out in the presence of 1 molar proportion of ethyl methyl sulphide.[16] The results are recorded in Table 1.

Electrolytic Reduction of Nitro-arginine and Related Peptides.—The apparatus is shown in the Figure. The membrane (A) was a circle of Viscocel sheet, the ends of which were secured above the level of the solution. The anode was of smooth platinum (0·64 cm.² area), and the cathode was a pool of purified mercury (7 cm.² area). The electrolyte (B) was N-sulphuric acid; the nitro-compound (*ca.* 400 mg.) was dissolved in the catholyte, which was stirred magnetically, and the cell was cooled externally by water. Current (0·2 amp.) was supplied by a 6v accumulator. In early experiments the saturated calomel electrode (C) was used to measure the cathode potential, but control of this potential was not necessary in the cases examined. The time required for reduction was determined by paper electrophoresis of a sample of the catholyte, at pH 11·5 or 6·0; the increase in basicity and appearance of a Sakaguchi reaction enabled a clear distinction between the nitroarginine and arginine derivatives to be made. It was important to stop the reduction as soon as nitro-compound had disappeared. The catholyte was then removed by means of a pipette, the cell was washed out with 5% acetic acid, and the combined solutions were passed down a column containing 4 g. of Dowex-3 acetate resin. The

[16] Guttmann and Boissonnas, *Helv. Chim. Acta*, 1958, **41**, 1852.

eluate, which should be free from sulphate, was evaporated to dryness below 40°/0·05 mm. In some cases, the residue was then analytically pure; any further purification required is indicated in Table 2. In all cases except that of L-arginyl-L-leucine acetate, the yield given is for analytically and chromatographically (BWA and PW) pure product, with the constants stated.

N^{α}-*Benzyloxycarbonyl*-L-*arginylglycine*.—N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginylglycine (200 mg.) was dissolved in tetrahydrofuran (2 ml.) and N-sulphuric acid (2 ml.), and the solution was reduced electrolytically in the usual way, at 0·2 amp. for 3 hr. The solution was passed down a column of Dowex-3 acetate resin, and the filtrate was evaporated to dryness. The residue was taken up in methanol and precipitated with ether. Recrystallisation from methanol–water gave colourless needles of the *benzyloxycarbonyl-dipeptide* (110 mg., 62%), m. p. 141·5—142·5°, $[\alpha]_D^{22}$ −7° (*c* 1·0 in 95% acetic acid) (Found, after drying at 110°/0·05 mm.: C, 49·9; H, 6·8; N, 18·3. $C_{16}H_{23}N_5O_5,H_2O$ requires C, 50·1; H, 6·6; N, 18·3%).

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