New Methods in Peptide Synthesis. Part IV.[1] N → S Transfer of *N*-*o*-Nitrophenylsulphenyl Groups in Cysteine Peptides[2]

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When the *N*-*o*-nitrophenylsulphenyl group is removed from cysteine peptides by means of hydrogen chloride in methanol or non-polar solvents, or by means of acids in aqueous methanol or acetone, an N → S transfer of the *o*-nitrophenylsulphenyl-group takes place, to give the corresponding *S*-o-nitrophenylsulphenyl derivative. Even in alkaline solution transfer of the *o*-nitrophenylsulphenyl group from the α-amino-group to the thiol can occur to some extent.

THE *o*-nitrophenylsulphenyl group is finding an increasingly wide application in the protection of the α-amino-group during peptide synthesis.[3,4] Some new *N*-*o*-nitrophenylsulphenyl derivatives of amino-acids and their esters are here described. Most of them were prepared according to published procedures.[3,4] *N*-*o*-Nitrophenylsulphenyl-L-histidine, *N*-*o*-nitrophenylsulphenyl-L-

[1] Part III, G. C. Stelakatos, A. Paganou, and L. Zervas, *J. Chem. Soc.* (*C*), 1966, 1191.

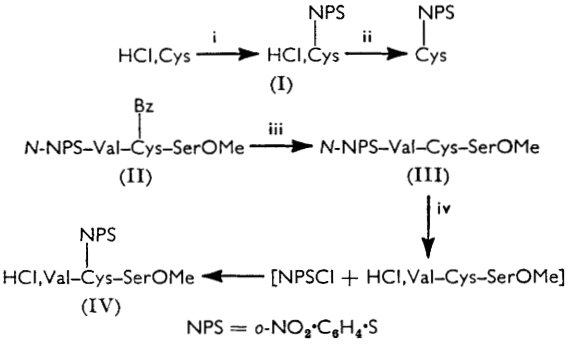
[2] Presented in part as L. Zervas, I. Photaki, C. Yovanidis, J. Taylor, I. Phocas, and V. Bardakos, "Peptides: Proceedings of the Eighth European Symposium," ed. H. C. Beyerman, North-Holland Publishing Co., Amsterdam, in the press.

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

[4] L. Zervas and Ch. Hamalidis, *J. Amer. Chem. Soc.*, 1965, **87**, 99.

arginine and their methyl esters were prepared by a modified method. The *N*-*o*-nitrophenylsulphenyl derivatives of the basic amino-acids and of their esters are mono-derivatives, presumably α-substituted.

Sulphenyl chlorides also react with the thiol group of L-cysteine both in alkaline solution [4,5] and, as we have observed with *o*-nitrophenylsulphenyl chloride, in acidic solution. The mixed disulphide, *S*-*o*-nitrophenylsulphenyl cysteine (I),† thus formed is not stable but rearranges very quickly, especially in alkaline solution, to give, among other products, di-*o*-nitrophenyl disulphide. Therefore, when the *N*-*o*-nitrophenylsulphenyl-group is removed from cysteine peptides, *e.g.*, (III), by means of hydrogen chloride in methanol or non-polar solvents, the chloride generated reacts with the free thiol group to give, in excellent yield, the corresponding *S*-*o*-nitrophenylsulphenyl derivative (IV).



Reagents: i, NPS–Cl, $HCONMe_2$; ii, CH_3CO_2Na; iii, (a) MeONa–MeOH, (b) CH_3CO_2H; iv, 2HCl, MeOH, $MeCO_2Et$, etc.

This transfer of the *o*-nitrophenylsulphenyl group is an N ⟶ S migration and the reaction also takes place when the *N*-*o*-nitrophenylsulphenyl group is removed by acids, *e.g.*, toluene-*p*-sulphonic acid, in solvents such as aqueous alcohol or acetone. The removal of the *N*-*o*-nitrophenylsulphenyl group in such solutions of *N*-*o*-nitrophenylsulphenyl peptides, even with one equivalent of acid, has already been described, and the subsequent uptake of the *o*-nitrophenylsulphenyl group by the thiol group of cysteine may be explained on the basis of the recent work of Kessler and Iselin.[7] These authors report that thiols such as thiophenol, in the presence of acetic acid, remove the *N*-*o*-nitrophenylsulphenyl-group as phenyl *o*-nitrophenyl disulphide and its rearrangement products. In the case of peptide (III), the peptide itself acts as the thiol. This means that during the preparation of cysteine peptides using *N*-*o*-nitrophenylsulphenyl derivatives of amino-acids and *S*-protected cysteines, the *N*-*o*-nitrophenylsulphenyl group must be removed before the liberation of the free thiol or after its oxidation.

Even in alkaline solution transfer of the NPS-group from the α-amino-group to the thiol can occur to some extent. Thus, in the case of *N*-*o*-nitrophenylsulphenyl peptides *e.g.*, (II), the methanolysis of the *S*-acyl group with methanolic sodium methoxide (20—30 min.) causes the disappearance of some of the free thiol groups. An explanation of this fact could be that the sodium thiolate of the peptide formed during methanolysis removes the *N*-*o*-nitrophenylsulphenyl group. Sodium benzene-

$$NPS\text{–}NHCHMe\cdot CO_2CH_2Bz \xrightarrow{PhSNa} H_2NCHMe\cdot CO_2H$$

thiolate removes the *N*-*o*-nitrophenylsulphenyl group from *N*-*o*-nitrophenylsulphenyl-L-alanine phenacyl ester at room temperature to a great extent, and indeed more quickly than thiophenol–acetic acid.[7] Therefore better yields are obtained by reducing the time of methanolysis of *N*-*o*-nitrophenylsulphenyl-*S*-acyl cysteine peptides (II) to *ca.* 10 min., rapid extraction of the peptide thiol after the acidification of the solution with acetic acid, and subsequent repeated washing of the extract with water in order to remove the acetic acid.

The susceptibility of the *N*-*o*-nitrophenylsulphenyl group to sodium benzenethiolate, a specific reagent for removal of the phenacyl ester group,[1,8] means that this thiolate cannot be used for the selective removal of phenacyl groups from *N*-*o*-nitrophenylsulphenyl-amino-acid phenacyl esters.

EXPERIMENTAL

Where reaction mixtures were non-aqueous, solvents were dried before use. Evaporations were carried out under reduced pressure at 35—40°. Compounds were dried *in vacuo* at room temperature (P_2O_5) with the exception of compounds (I), (III), and (IV), which were dried *in vacuo* at 95° (P_2O_5) for 12 hr. All substances were chromatographically pure (t.l.c.[9] on Kieselgel G). For substances with a free amino-group, the solvent systems n-butanol–acetic acid–pyridine–water (30 : 6 : 20 : 24) and 3,3-dimethylpropan-1-ol–pyridine–water–diethylamine (10 : 10 : 7 : 7 : 0·3) were used and the chromatograms were developed with ninhydrin. For *N*-*o*-nitrophenylsulphenyl derivatives the systems used were chloroform–carbon tetrachloride–methanol (6 : 3 : 1) and toluene–pyridine–acetic acid (80 : 10 : 1) and development was done with iodine. Microanalyses were done by Dr. H. Mantzos, Analytical Laboratory of the Royal Hellenic Research Foundation.

N-*o*-*Nitrophenylsulphenylamino-acids and their Esters*.—The above compounds are listed in the Table; most of them have been prepared according to one of the already described methods,[3,4] and relevant procedures are indicated for each derivative in the Table. Modified procedures for the preparation of *N*-*o*-nitrophenylsulphenyl derivatives of histidine, arginine, and their methyl esters are described below.

N-*o*-*Nitrophenylsulphenyl*-L-*histidine methyl ester*. For 1 mole of L-histidine methyl ester dihydrochloride 2 moles

† 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.[6]

[5] S. Sakakibara and H. Tani, *Bull. Chem. Soc. Japan*, 1956, **29**, 85.

[6] "Peptides: Proceedings of the Fifth European Symposium," ed. G. T. Young, Pergamon, Oxford, 1963, p. 261.

[7] W. Kessler and B. Iselin, *Helv. Chim. Acta*, 1966, **49**, 1330.

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

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

of *o*-nitrophenylsulphenyl chloride and 4 moles of triethylamine were used.

N-o-*Nitrophenylsulphenyl-L-arginine methyl ester hydrochloride*. Methanol was used as solvent. After the addition of the reactants [L-arginine methyl ester dihydrochloride (1 mole), *o*-nitrophenylsulphenyl chloride (1 mole), and triethylamine (2 moles)], the reaction mixture was stirred for 10 min. and filtered. The filtrate was evaporated to dryness, a little water was added, and the mixture was left for several hours in the refrigerator; the product separated out.

N-o-*Nitrophenylsulphenyl-L-histidine*. The solvent used was methanol–water (1 : 1; 50 ml. for 0·02 mole of amino-acid) and the base was triethylamine (2 equiv. added at the beginning of the reaction). The *o*-nitrophenylsulphenyl chloride was added in portions to the stirred mixture over a period of 10 min. and stirring was continued for a further 30 min. The precipitated N-*substituted histidine* was filtered off and washed with aqueous methanol (2 × 10 ml.) and water (30 ml.). The crude product was triturated with methanol (15 ml.) and left at room temperature for 24 hr. It was then filtered off and washed with methanol (5 ml.).

N-o-*Nitrophenylsulphenyl-L-arginine* was prepared as described for the corresponding histidine derivative except that 85% methanol was used, and the temperature was kept at 10°. The reaction mixture was allowed to stand for several hours in the refrigerator, and the product filtered off and washed with methanol.

Removal of the Nitrophenylsulphenyl Group and Phenacyl Ester Group from N-o-*Nitrophenylsulphenyl-L-alanine Phenacyl Ester*.—To a solution of *N-o*-nitrophenylsulphenyl-L-alanine phenacyl ester (0·72 g., 0·002 mole) in dry dimethylformamide, sodium benzenethiolate (0·532 g., 0·004 mole) was added. After 30 min.* in the dark at room temperature water (50 ml.) was added, and the mixture was acidified with N-sulphuric acid and extracted with ether–ethyl acetate (1 : 1). The organic layer was washed repeatedly with water, dried (Na_2SO_4), and concentrated. The residue was reprecipitated from ethyl acetate with light petroleum (b. p. 40—70°) to give a substance, m. p. 170—180°. This product was triturated 3 times with ethanol at 50°; each trituration was followed by decantation, and finally the residue was filtered off, washed with ethanol, and dried. The product 150 mg. (50%), m. p. 192—198°, was di-*o*-nitrophenyl disulphide (identified by chromatographic behaviour and i.r. spectrum).

S-o-*Nitrophenylsulphenyl-L-cysteine* (I).—To a cold solution of anhydrous L-cysteine hydrochloride[10] (1·6 g., 0·01 mole) in pure anhydrous dimethylformamide (10 ml.) NPS-chloride (1·9 g., 0·01 mole) was added. After 1 hr. in the refrigerator and 1 hr. at room temperature, the hydrochloride of (I) precipitated out. Addition of a cold aqueous solution of sodium acetate (1·6 g., in 40 ml.) to this mixture resulted in the precipitation of S-o-*nitrophenylsulphenyl-L-cysteine* (2·1 g., 77%), which was washed with cold water and kept in a desiccator (P_2O_5), m. p. 168° (decomp.), $[\alpha]_D^{25}$ −72° (*c* 1 in methanol) (Found: C, 39·2; H, 3·8; N, 10·5; S, 22·9. $C_9H_{10}N_2O_4S_2$ requires C, 39·4; H, 3·7; N, 10·2; S, 23·4%).

N-o-*Nitrophenylsulphenyl-L-valyl-L-cysteinyl-L-serine Methyl Ester* (III).—*N-o*-Nitrophenylsulphenyl-L-valyl-*S*-benzoyl-L-cysteinyl-L-serine methyl ester[11] was subjected to methanolysis as already described,[11] with the modification that the time of methanolysis was reduced to 8—10 min.† The solution was then cooled immediately to −10°, acidified with acetic acid (2 ml.) in cold water‡ (20 ml.), and extracted first with peroxide-free ethyl acetate–ether (1 : 1) and then with ether alone. The combined extracts were washed with water, dilute potassium hydrogen carbonate, and again with water, dried (Na_2SO_4) and evaporated to dryness at room temperature. The

TABLE

N-o-Nitrophenylsulphenyl (NPS) derivatives of amino-acids and amino-acid esters

N-o-Nitrophenylsulphenyl-derivatives of	Recrystallised from	Yield (%)	M. p.	$[\alpha]_D$	Found (%) C	H	N	S	Cl	Formula	Calc. (%) C	H	N	S	Cl
L-glutamic acid[a]	AcOEt–light petroleum	71	132—133°	−84·5°			9·3	10·65		$C_{11}H_{12}N_2O_6S$			9·3	10·7	
L-glutamic acid DCHA salt[b,c]	MeOH–AcOEt	70	178—179	−13·8[g]			8·5	4·8		$C_{35}H_{58}N_4O_6S$			8·45	4·8	
L-aspartic acid DCHA salt[b]	MeOH–ether	50	180—181	−19·0[g]	63·0	8·95	8·8			$C_{34}H_{56}N_4O_6S$	62·9	8·7	8·6		
L-threonine[a]	AcOEt	50	144—145[h]	−130·8[f,h]											
L-alanine phenacyl ester[d]	MeOH	50	108—110	−127·3[i]	56·6	4·6	8·0	9·0		$C_{17}H_{16}N_2O_5S$	56·65	4·5	7·8	8·9	
L-histidine methyl ester[e]	AcOEt	88	134—136	+64·1[j]	48·4	4·1	17·5	10·0		$C_{13}H_{14}N_4O_4S$	48·4	4·4	17·4	9·9	
L-arginine methyl ester hydrochloride[e]	Water	63	75		41·5	5·4	8·45	8·4	9·5	$C_{13}H_{19}N_5O_4S,HCl$	41·35	5·3	8·5	8·5	9·4
L-histidine[e]		47	185—188	−6·9[j]	46·8	3·85	18·6	10·1		$C_{12}H_{12}N_4O_4S$	46·7	3·9	18·2	10·4	
L-arginine[e]		80	164	−10·8[k]	44·1	5·45	21·6	9·5		$C_{12}H_{17}N_5O_4S$	44·0	5·2	21·4	9·8	

DCHA = Dicyclohexylamine.

[a] Prepared by method A, ref. 3. [b] Prepared by method B, ref. 3. [c] Prepared by addition of DCHA to an ethyl acetate solution of the corresponding free acid. [d] Prepared by known procedure[3,4] starting from L-alanine phenacyl ester hydrobromide.[1] [e] See Experimental section. [f] *c* 2 in dimethylformamide. [g] *c* 3 in methanol. [h] Previously reported[3] data, m. p. 138—141°, $[\alpha]_D$ −111·6°. [i] *c* 1·6 in ethyl acetate. [j] *c* 1 in dimethylformamide. [k] *c* 2·5 in dimethylformamide.

* Chromatography of the reaction mixture after 30 min. incubation with benzenethiolate showed the presence of both *N-o*- nitrophenylsulphenyl alanine and alanine as well as a small amount of the original *N-o*-nitrophenylsulphenylalanine phenacyl ester indicating that the *N-o*-nitrophenylsulphenyl group is split but only partially under the conditions used.

† Titration with iodine after acidification with acetic acid (2 ml.) in oxygen-free water (3 ml.) showed 90% free thiol. If the time of methanolysis was increased to 15 min. only 58% free thiol could be detected and after 20 min. the percentage was reduced to 37%.

‡ When handling compounds bearing free thiol the water used was always free of oxygen.

[10] M. Bergmann and G. Michalis, *Ber.*, 1930, **63**, 987.

[11] L. Zervas, I. Photaki, A. Cosmatos, and D. Borovas, *J. Amer. Chem. Soc.*, 1965, **87**, 4922.

residue was dissolved in ethyl acetate and reprecipitated with light petroleum (b. p. 40—70°) to give the compound (III) (0·42 g., 88%), m. p. 148—150° (from ethyl acetate), $[\alpha]_D^{25}$ −20·1° (*c* 2·3 in dimethylformamide) [lit.,[11] m. p. 148° $[\alpha]_D^{20}$ −17·2° (*c* 2·5 in dimethylformamide)].

L-*Valyl*-S-O-*nitrophenylsulphenyl*-L-*cysteinyl*-L-*serine Methyl Ester Hydrochloride* (IV).—To a solution of *N*-*o*-nitrophenylsulphenyl-L-valyl-L-cysteinyl-L-serine methyl ester (0·24 g., 0·005 mole) in absolute methanol (3 ml.), methanolic N-HCl (1 ml.) was added. The solution was left at room temperature for 15 min. and anhydrous, peroxide-free ether was added; the S-NPS-*tripeptide ester hydrochloride* (0·2 g., 77%) precipitated out, m. p. 187° (from methanol–ether), $[\alpha]_D^{25}$ −60·1° (*c* 2 in dimethylformamide) (Found: C, 42·4; H, 5·6; Cl, 6·95; N, 10·8; S, 12·4. $C_{18}H_{27}ClN_4O_7S_2$ requires C, 42·3; H, 5·3; Cl, 6·9; N, 10·9; S, 12·6%).

To prepare the corresponding tosylate, toluene-*p*-sulphonic acid hydrate (0·38 g.) was added to a solution of (III) (0·474 g., 0·001 moles) in 90% methanol, or acetone (10 ml.), and the mixture was shaken for 1 hr. It was concentrated to 3—4 ml., ethyl acetate and ether were added, and L-*valyl*-S-O-*nitrophenylsulphenyl*-L-*cysteinyl*-L-*serine methyl ester tosylate* was obtained, (0·34 g., 60%), m. p. 144—147° (from methanol–ether), $[\alpha]_D^{27}$ −25·2° (*c* 1·8 in dimethylformamide) (Found: C, 46·2; H, 5·7; N, 9·0; S, 15·1. $C_{25}H_{34}N_4O_{10}S_3$ requires C, 46·4; H, 5·3; N, 8·7; S, 14·9%).

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