N-Quaternary Compounds. Part XXIX.[1] Selective Removal of the *N*-Chloroacetyl Group in Peptides

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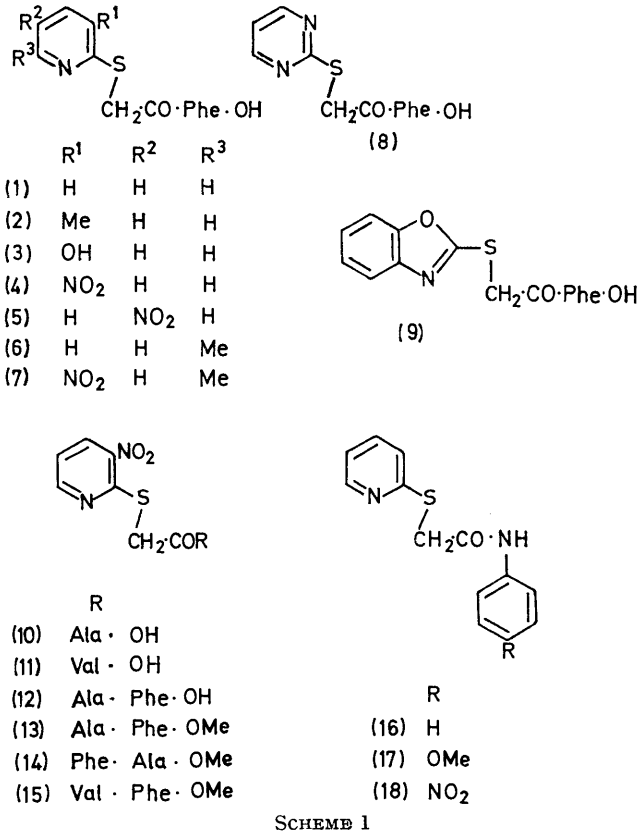
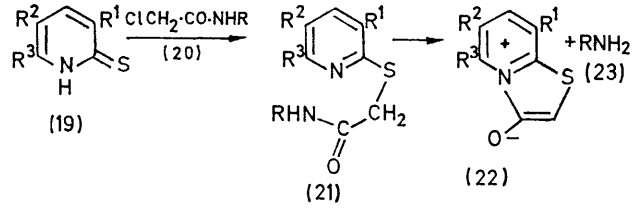
The *N*-chloroacetyl group can be removed from amino-acids and dipeptides in two steps as follows. The chloroacetamide and a pyridine-2-thione are initially condensed in aqueous sodium hydrogen carbonate. The condensation product in cold trifluoroacetic acid undergoes cyclisation with concomitant liberation of the amine. Effects of substituents in the pyridine system and in the amine have been studied. The best reagent was found to be 3-nitropyridine-2-thione.

RECENTLY we reported that alanine can be *N*-acylated by a thiazolo[3,2-*a*]pyridinium-3-olate and that the adduct is readily cleaved in trifluoroacetic acid (TFA) to give the original reactants without racemisation of the amino-acid.[2] This observation suggested the possibility of selective removal of an *N*-halogenoacetyl group, used as protecting group in peptide synthesis, by treatment with a thiolactam. Hitherto reported methods for the removal of a such group require more vigorous reaction conditions. Thus, heating *N*-chloroacetyl peptides with *o*-phenylenediamine leads to intramolecular aminolysis of the initially formed *N*-alkylated phenylenediamine.[3] A recent report described the selective removal of the chloroacetyl group by reaction with thiourea in ethanol followed by heating in aqueous solution; this leads to an intramolecular amidinolysis of the intermediate *S*-substituted thioformamidine.[4] The method has been improved by the use of *N*-substituted thioureas.[5] In our studies using pyridine-2-thiones and related compounds we have investigated substituent effects in the pyridine system, the nature of the heterocycle, and the influence of the amine system on the cyclisation which leads to amine liberation. The required model compounds (Scheme 1) were prepared as shown in Scheme 2 by condensation of chloroacetamides (20) with pyridine-2-thiones (19) in aqueous sodium hydrogen carbonate. The pyrimidine (8) and the benzoxazole (9) analogues were prepared similarly. As shown by chromatography, the conversion into the required product is almost quantitative. Most condensation products crystallised exceptionally well, especially those derived from 3-nitropyridine-2-thione, including the dipeptide esters (13)—(15). *N*-Chloroacetyl-L-alanyl-L-phenylalanine, required for the preparation of (12), was available by application of the mixed anhydride method to *N*-chloroacetyl-L-alanine and L-phenylalanine. The *N*-chloroacetyl dipeptide ester intermediates for the preparation of compounds (13)—(15) were prepared similarly from the respective L-amino-acid precursors by use of dicyclohexylcarbodiimide as the condensing agent.



SCHEME 1



a; $R^1 = R^2 = R^3 = H$
b; $R^1 = Me$, $R^2 = R^3 = H$
c; $R^1 = OH$, $R^2 = R^3 = H$
d; $R^1 = NO_2$, $R^2 = R^3 = H$
e; $R^1 = H$, $R^2 = NO_2$, $R^3 = H$
f; $R^1 = R^2 = H$, $R^3 = Me$
g; $R^1 = NO_2$, $R^2 = H$, $R^3 = Me$

SCHEME 2

Rate studies for the intramolecular aminolysis were conducted in TFA at 20 °C (Figure). In the case of the amino-acid derivatives, which were all of the L-configuration, the progress of the reaction was conveniently

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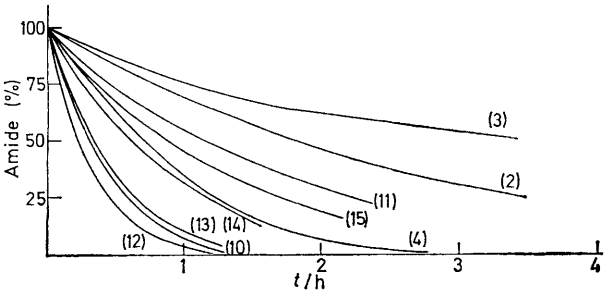
followed by the change in optical rotation at the sodium D-line. In a few cases the amino-acid liberated was isolated and was found to have suffered no or very little racemisation. The presence of the thiazole (22) in the TFA solution did not interfere to any extent with the specific rotation of the amine liberated. Alternatively



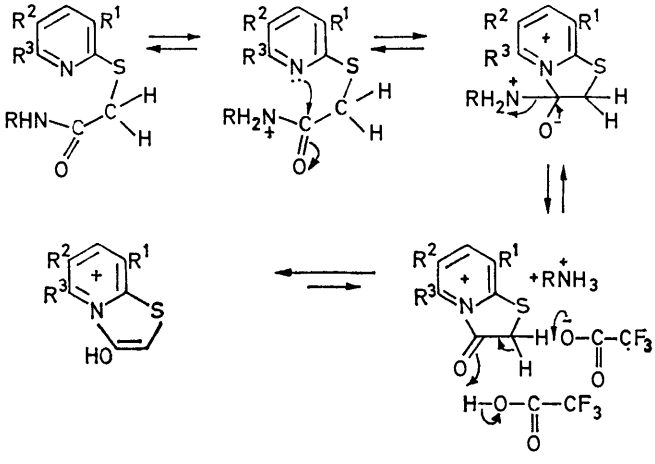
Cleavage of optically active *N*-(2-pyridylthio)acetyl-amines in TFA at 20 °C measured by observing the sodium D-line rotation

the progress of the reaction could be studied by n.m.r. as described later for the anilides (16)—(18).

The effects of some substituents in the pyridine ring were first investigated by use of the phenylalanine series (1)—(7). In the unsubstituted pyridine (1) an equilibrium position is established after some time between the amide (1) on the one side and the thiazole (22) and the amine (23) on the other. With a 3-methyl (2) or a 3-hydroxy- (3) group the reaction is complete after 6 and 10 h, respectively. The rate is still greater (2·5 h) with the 3-nitro-group (4), and the 5-nitro-isomer (5) does not react. The nitro-group in both these isomers would be expected to have a similar weakening effect on the nucleophilicity of the pyridine nitrogen atom. The second nitrogen atom in the pyrimidine (8) should also exert a similar electronic effect; like the benzoxazole (9) it did not react under these conditions. The major effect of the 3-substituent is therefore a steric one. Presumably the coplanar 3-nitro-group will favour conformations of the *S*-side-chain in which the carbonyl carbon atom is close to the ring nitrogen atom. As expected, the cyclisation is sensitive to non-bonded interaction between the carbonyl side-chain and a 6-substituent in the pyridine ring. Thus the 6-methyl derivative (6) did not react nor was the influence of the 3-nitro-group in (7) sufficient for the reaction to proceed.

Having found the 3-nitro-derivative (4) to be the most reactive, we used 3-nitropyridines in all the subsequent work. The aminolysis rate (Figure) for the valine (11), the phenylalanine (4), and the alanine (10) increases in this order in accord with the decrease in steric interaction from the substituents on the β-carbon atom of the amino-acid. The steric influence of the β-substituent in the amine is further illustrated by the alanylphenylalanine dipeptide (12), which was deacylated at about the same rate as the alanine (10). The esters (13)—(15) behave in the same way, the reaction times being 70 min, 3·5 h, and 4 h, respectively; *cf.* 60 min, 2·5 h, and 4 h for the amino-acids (10), (4), and (11), respectively.

The anilides (16)—(18) were prepared for the study of the effects of electronic changes in the amine system on the cyclisation reaction, as the *para*-substituents do not exert a steric effect. N.m.r. was used to determine the reaction rate in TFA by observation of the disappearance of the methylene proton signals at τ 6·0 and the appearance of a new signal at τ 2·6 due to the C-2 proton in the thiazole ring. The reaction time for the *para*-nitro-derivative (18) was 15 min, that for the anilide (16) *ca.* 2 min, and that for the methoxy-derivative (17) *ca.* 1 min. These data are interpreted to mean that protonation of the amide group, which is favoured by an electron-donating *p*-substituent, is the rate-determining factor. The importance of having an acid present which is strong enough for protonation was also suggested by the failure of attempts to cyclise (4) in acetic acid solution. Very little reaction was observed after 4 days. A reaction sequence is suggested in Scheme 3.



SCHEME 3

EXPERIMENTAL

N.m.r. spectra were recorded on a Varian A-60A instrument and t.l.c. was run on silica gel 254 in the system BuOH–EtOH–NH_3–H_2O (4 : 1 : 2 : 1).

The N-(2-Pyridylthio)acetyl Derivatives (1)—(11).—A solution of the *N*-chloroacetyl-L-amino acid (2·5 × 10^{-3} mol), the pyridine-2-thione, or pyrimidine-2-thione, or 2-mercaptobenzoxazole (2·5 × 10^{-3} mol), and sodium hydrogen carbonate (1 g) in water (15 ml) was heated at 40—50 °C until t.l.c. showed the reaction to be complete (2—3 h). Acidification of the solution to pH 4 with HCl led to slow precipitation of the product. Further data are given in the Table.

N-(3-Nitro-2-pyridylthio)acetyl-L-alanyl-L-phenylalanine (12).—The required *N*-chloroacetyl peptide was synthesised from *N*-chloroacetylalanine and phenylalanine by the mixed anhydride method. The peptide thus obtained (1·25 × 10^{-3} mol), 3-nitropyridine-2-thione (1·25 × 10^{-3} mol), and sodium carbonate (1 g) in water (25 ml) were heated at 40 °C for 3 h. Acidification to pH 4 with HCl led to precipitation of the title compound. Further data are given in the Table.

Syntheses of the N-(3-*Nitro*-2-*pyridylthio*)*acetyl Dipeptide Methyl Esters* (13)—(15).—Dicyclohexylcarbodi-imide ($1{\cdot}1 \times 10^{-2}$ mol) was added to a solution of the *N*-chloroacetyl-L-amino acid (10^{-2} mol) dissolved in tetrahydrofuran (50 ml) at −5 °C. After 2 h at this temperature the L-amino-acid methyl ester dissolved in chloroform (50 ml) was added and the resultant solution was kept at 0 °C overnight. Water (20 ml) and acetic acid (2 ml) were then added and the precipitated dicyclohexylurea was filtered off after 1 h at room temperature. The organic phase was then separated, the aqueous phase was extracted with ethyl acetate, and the combined organic solutions were dried (Na_2SO_4) and evaporated. The *N*-chloroacetyl dipeptide methyl ester thus obtained was added to a mixture of 3-nitropyridine-2-thione (10^{-2} mol) and sodium hydrogen carbonate (3 g) in acetone–water (1 : 1; 50 ml). The reaction solution was heated at 40 °C for 2 h before cooling and extraction of the product into ethyl acetate, from which the product was isolated after drying and evaporation. Further data are given in the Table.

N-(3-*Nitro*-2-*pyridylthio*)*acetylanilines* (16)—(18).—A solution of the *N*-chloroacetylaniline (10^{-2} mol), 3-nitropyridine-2-thione (10^{-2} mol), and sodium hydrogen carbonate (2 g) in acetone–water (1 : 1; 50 ml) was heated at 50 °C for 3 h. Most of the acetone was then evaporated off before addition of water (25 ml), which led to slow precipitation of the product. Further data are given in the Table.

Polarimetry.—The rate of cleavage of compounds (1)—(15) in a 1% solution in TFA at 20 °C was studied by recording the change in the sodium D-line rotation.

Preparative studies are described below.

Cleavage of N-(2-*Pyridylthio*)*acetyl*-L-*phenylalanine* (1).—(1) (10^{-3} mol) dissolved in TFA (5 ml) was kept at 20 °C. After 1 day the chromatogram, which showed the presence of (1), phenylalanine, and thiazolo[3,2-*a*]pyridinium-3-olate, did not change further. The solution was then evaporated, water (10 ml) was added to the residue, the pyridine derivative was extracted into chloroform, and the aqueous solution was evaporated to leave the white crystalline TFA salt of phenylalanine, $[\alpha]_D^{20}$ −10·7° (1% in H_2O). The TFA salt of reference L-phenylalanine gave $[\alpha]_D^{20}$ −10·8° (1% in H_2O).

Cleavage of N-(3-*Nitro*-2-*pyridylthio*)*acetyl*-L-*phenylalanine* (4).—A solution of compound (4) (10^{-3} mol) in TFA (5 ml) was left at 20 °C for 4 h, then evaporated at reduced pressure. The residue was triturated with water (15 ml) and the yellow 8-nitrothiazolo[3,2-*a*]pyridinium-3-olate (22) formed was extracted into chloroform. The colourless, aqueous solution was evaporated to leave the L-phenylalanine TFA salt (83%), $[\alpha]_D^{20}$ −10·5° (1% in H_2O).

Cleavage of N-(3-*Nitro*-2-*pyridylthio*)*acetyl*-L-*alanine* (10).—The reaction as before was run in TFA at room temperature for 2 h and gave L-alanine TFA salt in 99% yield, $[\alpha]_D^{20}$ +6·0° (1% in H_2O); *cf.* $[\alpha]_D^{20}$ +6·1° for reference alanine TFA salt.

Cleavage of N-(3-*Nitro*-2-*pyridylthio*)*acetyl*-L-*valine* (11).—Reaction in TFA for 6 h gave L-valine TFA salt in 95% yield, $[\alpha]_D^{20}$ +31·3° (1% in H_2O). The specific rotation is the same as that of the reference compound.

Cleavage of N-(3-*Nitro*-2-*pyridylthio*)*acetyl*-L-*alanyl*-L-*phenylalanine* (12).—The reaction was run as before for 2 h. L-Alanyl-L-phenylalanine thus obtained had $[\alpha]_D^{20}$ +36·3° (1% in H_2O at pH 5) (lit.,[6] 37·5°).

Compounds (3),[2] (19b),[7] (19d),[8] (19e),[8] (19f),[9] and (19g) [10] were synthesised according to literature methods.

Properties of products

Compd.	M.p. (°C)	Solvt. for recryst.	$[\alpha]_D$ 20 (°) (1% in TFA)	Yield (%)	Formula	Found (%) C	Found (%) H	Found (%) N	Calc. (%) C	Calc. (%) H	Calc. (%) N
(1)	152—154	H_2O	+92·7	85	$C_{16}H_{16}N_2O_3S$	61·0	5·35	8·5	60·75	5·1	8·85
(2)	187—188	EtOH–H_2O	+100·2	94	$C_{17}H_{18}N_2O_3S$	62·15	5·4	8·2	61·8	5·5	8·5
(4)	200—210	EtOH	+166·5	83	$C_{16}H_{15}N_3O_5S$	53·45	4·05	11·55	53·2	4·2	11·65
(5)	195—197	EtOH	+109·4	69	$C_{16}H_{15}N_3O_5S$	53·05	4·3	11·6	53·2	4·2	11·65
(6)	114—116	PhH	−70·6 *	91	$C_{17}H_{18}N_2O_3S$	62·0	5·4	8·4	61·8	5·5	8·5
(7)	154—156	EtOH–H_2O	+129·9	91	$C_{17}H_{17}N_3O_5S$	54·7	4·35	11·25	54·4	4·55	11·3
(8)	154—155	H_2O	+27·7	89	$C_{15}H_{15}N_3O_3S$	56·75	4·75	13·4	56·95	4·45	13·3
(9)	190—192	EtOH–H_2O	−7·6 *	84	$C_{18}H_{16}N_2O_4S$	60·4	4·6	8·0	60·65	4·55	7·85
(10)	193—194	H_2O	−57·7	67	$C_{10}H_{11}N_3O_5S$	42·0	3·85	14·7	42·1	3·9	14·75
(11)	153—154	EtOH–H_2O	+29·7	90	$C_{12}H_{15}N_3O_5S$	45·8	4·9	13·5	46·0	4·85	13·4
(12)	189—191	EtOAc–PhMe	−77·6	93	$C_{19}H_{20}N_4O_6S$	52·9	4·85	12·95	52·75	4·65	12·95
(13)	168—170	EtOH	−8·5	45	$C_{20}H_{22}N_4O_6S$	54·0	5·25		53·8	4·95	
(14)	189—191	EtOH	+85·3	40	$C_{20}H_{22}N_4O_6S$	54·3	4·85		53·8	4·95	
(15)	180—182	EtOH–H_2O	+25·7	57	$C_{22}H_{26}N_4O_6S$	56·0	5·65		55·7	5·5	
(16)	146—147	EtOH–H_2O		90	$C_{13}H_{11}N_3O_3S$	54·2	3·95	14·45	53·95	3·85	14·5
(17)	143—145	EtOH		78	$C_{14}H_{13}N_3O_4S$	52·7	4·25	12·9	52·65	4·1	13·15
(18)	213—215	EtOAc		92	$C_{13}H_{10}N_4O_5S$	46·95	3·2	16·85	46·7	3·0	16·75

* Specific rotation determined in EtOH.

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