Facile Trisulphide Formation in the Thermolysis of *N,N'*-Diacetyl-L-cystine Bismethylamide, an Excellent Model for Protein-bound Cystine

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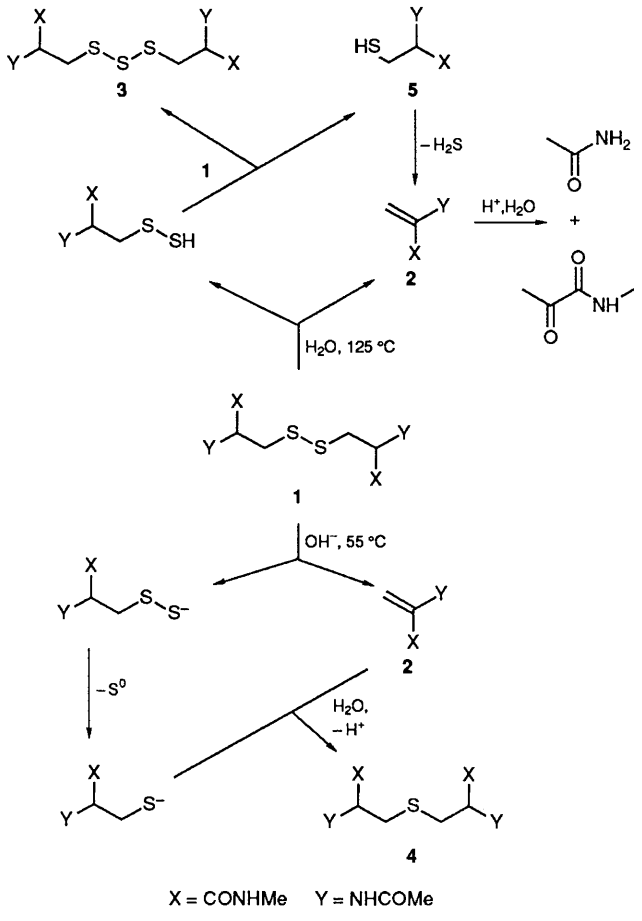
The thermolysis of *N,N'*-diacetyl-L-cystine bismethylamide not only displays the known chemistry of protein-bound cystine, but also draws attention to the facile formation of trisulphides and, when conducted under alkaline conditions, allows the isolation of the simplest α,β-dehydropeptide.

Protein-bound cystine is heavily implicated in the thermal-yellowing and fibre-weakening of wool keratin. Although extensive studies have shown that β-elimination in the side chain plays an important part in the degradation,[1,2] molecular explanations of the observed effects remain incomplete.[3] In seeking further elaboration by analysing the thermolysates of a close model, *N,N'*-diacetyl-L-cystine bismethylamide[4] **1**, we have found that trisulphide formation is predominant under neutral or acidic conditions. In alkali, however, the primary product is that expected from β-elimination, *N*-acetyldehydroalanine methylamide **2** which, unlike the parent amino acid,[5] is readily isolated. While the facile formation of *N,N'*-diacetylthiocystine bismethylamide **3** accords with the widespread intermediacy of thiocystine and thiocysteine derivatives in metabolic processes,[6] its possible significance for the chemical treatment of cystine-rich proteins in food[7] and wool[1] technologies has received little attention.

Thermolysis under initially neutral conditions was effected typically with compound **1** (1 mmol) and water (30 mmol) kept under nitrogen in a stainless steel tube held at 125 °C. (No reaction occurs in the absence of water.) Alkaline degradation was carried out at 55 °C in aqueous solution (0.4 mol dm^{-3}) buffered at pH 10.7 with sodium borate. Loss of **1** and formation of products was monitored by reversed phase HPLC (typically: SP, Spherisorb S5 ODS-2, 250 × 5 mm; MP, 4% aq. ACN; λ 215 nm) directly or, in the case of solid samples, after filtration of aqueous solutions. Products were isolated by preparative HPLC (typically: SP, Spherisorb S5 ODS-2, 250 × 20 mm; MP, 6% aq. ACN; λ 220 nm) after 70% loss of **1** (30 and 20 h under the neutral and alkaline conditions, respectively).

Both types of experiment produce deep-yellow thermolysates. That from the solid, initially neutral, substrate gives an acidic aqueous solution which slowly deposits elemental



Scheme 1

Table 1 Yields[a] of products after 25% thermolysis of *N,N'*-diacetyl-L-cystine bismethylamide

Conditions	2	3	4	5
Wet solid, 125 °C	0.02	0.35	0.02	0.12
Aqueous solution pH 10.7, 55 °C	0.70	0.06	0.24	0.05

[a] Molar ratio to substrate consumed.

sulphur and whose HPL chromatogram is dominated by a single well-retained product peak. This component was shown by fast atom bombardment (FAB) high resolution mass spectrometry (MH^+ = $C_{12}H_{23}N_4O_4S_3$ ± 0.000 05) and detailed spectroscopic comparison with **1** to be the labile trisulphide **3**, solutions of which decompose on standing to give elemental sulphur and some of the minor products seen in the HPLC of the original thermolysate. Compound **3** is formed so readily that it was always observed as a trace contaminant in the HPLC of freshly purified **1**. It is a minor component, however, in alkaline thermolysates, their HPL chromatograms showing clean production of **2** initially and then rapid growth of peaks due to the diastereoisomers of *N,N'*-diaceytllanthionine bismethylamide, **4**. Samples of **2** [M + NH_4^+ (CI) = $C_6H_{14}N_3O_2$ + 0.0001] and **4** [MH^+ (CI) = $C_{15}H_{23}N_4O_4S$ − 0.0001] isolated by preparative HPLC were fully characterized spectroscopically. Compound **4** and the minor product, *N*-acetylcysteine methylamide **5**, were confirmed by independent synthesis from a mixture of lanthionine diastereoisomers and by reduction of **1** with Zn/HCl, respectively.

The yields of the main products under the two conditions are compared in Table 1. The different distributions may be attributed principally to three factors: the high concentration of substrate in the wet solid, the lability of **2** at the low pH generated under these conditions[5] and, at high pH, the lability of persulphide, RSS^-[8] (Scheme 1).

The presence of lanthionine and other adducts of dehydroalanine in hydrolysates of wool protein degraded by alkali is well known and fully consistent with β-elimination in cystine residues.[3] The faithful reproduction of these reactions in the alkaline thermolysis of **1** confirms its suitability as a model for protein-bound cystine in respect of its chemical behaviour,† and invites consideration of the implications of the extreme ease with which trisulphides are formed at lower pH not only for the action of heat on cystine-rich proteins, but also for wider aspects of the biological chemistry of cystine residues.

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† The possible relevance of minor, but intensely yellow, products to the alkali-promoted yellowing of wool is under investigation.

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