HYDROLYSIS OF CYSTINE-*N*-PEPTIDE BONDS IN KERATIN BY HYDROCHLORIC ACID

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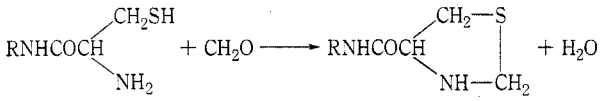
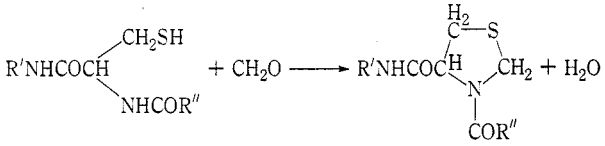
The reaction of certain mercaptans with formaldehyde may be used to determine them in mixtures with other mercaptans.[1] Dependence of the reaction on structure may be summarized as follows:

(*a*) Mercaptans with a free β-amino group react relatively rapidly to form a thiazolidine derivative.



(1)

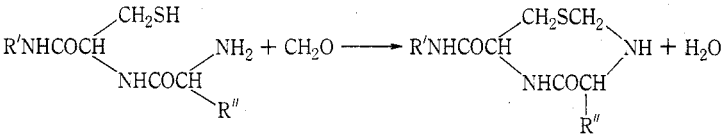
(*b*) The analogous reaction when the β-amino group is acylated (as in a peptide) is very slow in comparison.



(2)

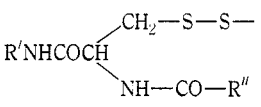
(*c*) If no nitrogen-containing group is present, reaction is much slower still.

(*d*) Although formation of 8-membered rings has not yet been investigated, this reaction is considered likely to be slow in comparison with (1), but more rapid than (2).



(3)

Hydrolytic cleavage of the cystine *N*-peptide bond in structure (I) may be demonstrated by reduction of the disulphide bond and titration of thiol groups with *o*-hydroxymercuribenzoic acid (HMB) in the presence of thiofluorescein as indicator before and after addition of formaldehyde.[1] Conditions must be chosen very carefully to cause reaction (1) to go to completion and to avoid reaction (3). In this paper results are corrected for occurrence of reaction (3) and for incompleteness of reaction (1).



(I)

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[1] Wroński, M., *Analyst*, 1963, **88**, 1048; 1964, **89**, 1065; and in press.

Experimental

A sample of keratin (0·5 g; weighed in a tube with both ends open) was introduced with the aid of a glass rod into each of a set of long, stoppered test tubes in an ultrathermostat. Each tube contained hydrochloric acid (7 ml) of chosen concentration (6N or 9N). The contents of the tubes were mixed from time to time until dissolution was complete (20–60 min), the time of hydrolysis being measured from the point of addition. At measured time intervals, to one of the test tubes was added a 50% w/w solution of potassium hypophosphite (KH_2PO_2; 0·3 ml) and sufficient 8N hydriodic acid to make the contents up to a standard volume (10 ml). After reduction of disulphide bonds was complete (*c.* 10 min at 90°), 4 or 5 pairs of aliquots were analysed at measured times as follows:

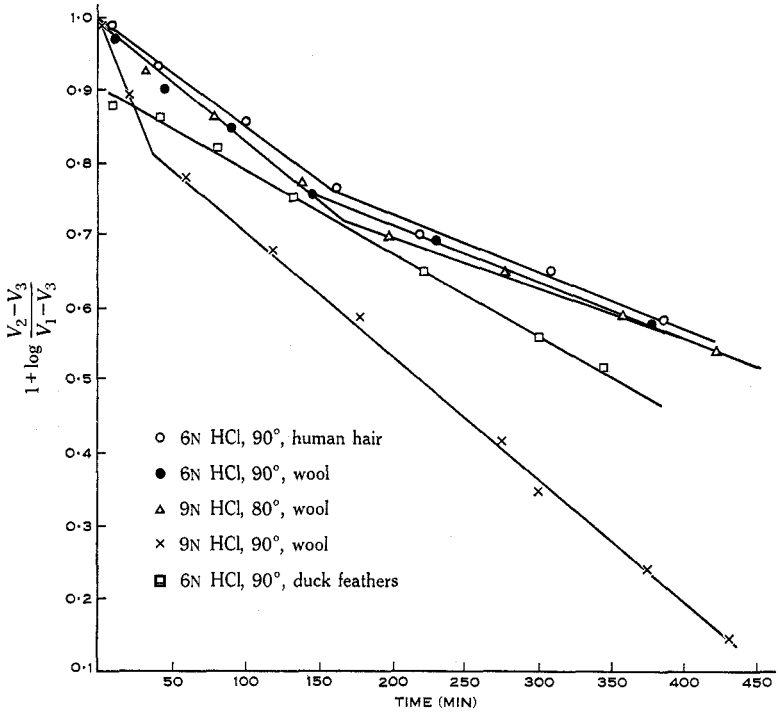

Fig. 1.—Rate of hydrolysis of the cystine *N*-peptide bond in keratin.

(1) An aliquot (1 ml) diluted with water (20 ml) was neutralized to pH 7 with 1N NaOH and an excess (1 ml) was added. The mixture was titrated with 0·0025N HMB in the presence of thiofluorescein, to disappearance of the blue colour. The titre, V_1 ml, corresponds to the total amount of thiols present, and was sensibly constant provided reduction was complete.

(2) A second aliquot (1 ml) was similarly diluted and neutralized, and an excess (2 ml) of 1N NaOH was added. The mixture was treated with 2% w/v formaldehyde solution (5 ml) and after standing for 1 min was titrated with 0·0025N HMB in the presence of thiofluorescein. The blue colour reappeared some time after completion of the titration but this was disregarded. The titre, V_2 ml, corresponds to the amount of thiols derived from cystine residues with unhydrolysed *N*-peptide bonds.

In one test tube of each set hydrolysis was allowed to proceed to completion and a titre, V_3, was obtained as for V_2 above. The value of V_3 represents a correction for consumption of HMB by the indicator and for incompleteness of reaction (1) under the conditions used. The value of V_3 was *c.* 9% of that of V_1.

For each test tube in a given set, values of the expression $(V_2-V_3)/(V_1-V_3)$ were extrapolated to the time of addition of hydriodic acid to give a value for use in the kinetic evaluation. This is based on the assumption that hydrolysis of the cystine *N*-peptide bonds is a first-order reaction.

$$-\mathrm{d[NHCO]}/\mathrm{d}t = k[\mathrm{NHCO}]$$

$$-kt = 2{\cdot}3\log([\mathrm{NHCO}]_t/[\mathrm{NHCO}]_0) \qquad (4)$$

The concentration of cystine *N*-peptide bonds before hydrolysis $[\mathrm{NHCO}]_0$, is given by the total cystine content, i.e. it is proportional to the corrected titre, V_1-V_3. The corresponding value after time t, $[\mathrm{NHCO}]_t$, is proportional to the corrected titre, V_2-V_3.

TABLE 1

RATE CONSTANTS FOR HYDROLYSIS OF KERATIN SAMPLES

Sample	Temp.	[HCl]	10^4k (min^{-1})	
			earlier line	later line
Wool	90°	6N	16	7·6
Wool	90	9N	54	16
Wool	80	9N	16	6·5
Human hair	90	6N	16	8·2
Duck feathers	90	6N	11	—

Results

Results obtained for merino wool, human hair, and duck feathers are shown in Figure 1 as plots of $\log(V_2-V_3)/(V_1-V_3)$ (extrapolated values) against time t. The points for wool seem to define two intersecting straight lines as do those for human hair, the earlier one in each case passing through the origin. In each case there are apparently at least two rate constants which correspond to two kinds of cystine *N*-peptide bonds. It may also be assumed that reaction of type (2) has been avoided in the analytical operations.

By contrast with the interestingly similar hydrolyses of human hair and wool, in the case of feathers the observed points fall on one straight line which does not pass through the origin. The slopes of the lines in Figure 1 are given in Table 1 as values of k.