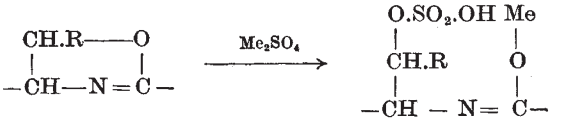
Oxazoline and Thiazoline Rings in Proteins

A STUDY of the *O*-methylation of wool and silk with methyl bromide and methyl sulphate showed the extent of methylation could not be entirely accounted for as esterification of free carboxyl groups and we suggested that methylation of activated peptide linkages also took place[1]. We have now obtained evidence suggesting the activated peptide linkages are possibly those which have undergone condensation with the side-chains of β-hydroxy acids, for example, serine and threonine. By this condensation, oxazoline rings may arise, which Bergmann and Miekeley[2], from studies of the reactions of *O*- and *N*-benzoylserine, have considered possible constituents of proteins.



We have now observed, in agreement with the above mechanism of methylation, that sulphuric acid is held firmly by wool methylated with methyl sulphate, and that methyl bromide, which would be less likely to open the oxazoline ring, gives less peptide methylation. We have also found that both acetyl and methyl groups can be introduced into wool, silk and collagen by the combined action of methyl alcohol and acetic anhydride. With wool, the extent of this new methylation is quantitatively similar to the peptide methylation obtained with methyl sulphate but not with either methyl bromide or iodide. For example, methyl sulphate introduced into different samples of the same wool 1·66 per cent CH_3; methyl bromide/iodide, 1·02 per cent CH_3; while acetic anhydride and methyl alcohol introduced 0·63 per cent CH_3. Hence, methyl sulphate introduced 0·64 per cent CH_3 to enolized peptide linkages as against 0·63 per cent CH_3 introduced by acetic anhydride and methyl alcohol. Methyl *p*-toluenesulphonate in methyl alcohol containing anhydrous potassium carbonate introduced 1·39 per cent CH_3 into this wool. Qualitative tests showed that some methylation of the tyrosine-hydroxyl may have occurred, but it is probable that cleavage of the oxazoline ring took place and both the peptide hydroxyl and the hydroxyl of the β-hydroxy acid were methylated.

Other evidence we have obtained suggests that free cysteine side-chains in reduced wool may form thiazoline rings in the same manner as serine side-chains form oxazoline rings, a possibility to which Linderstrøm-Lang and Jacobsen[3] have directed attention in their study of the reactions of methylthiazoline. Thus wool after maximum reduction with thioglycollic acid at *p*H 5[4] gives hydrolysates containing about half the original cystine-S as cysteine-S[5], but contrary to the findings of Harris and co-workers[4], we find that all the thiol groups in the reduced wool will not react with methylene iodide. After exposure to methylene iodide in buffer of *p*H 5, reduced wool ceases to give a nitroprusside reaction, but nevertheless, when hydrolysed yields 0·7 per cent of cysteine-S. A somewhat larger percentage of cysteine-S of the reduced wool fails to react with methylene iodide in buffer of *p*H 8. When oxygen is passed into buffer of *p*H 5 in which reduced wool is immersed, the cysteine-S is oxidized to cystine-S. If, however, the *p*H of the buffer is raised to 8, then about 0·7 per cent cysteine-S of the wool remains unoxidized, and again fails to give a nitroprusside reaction, unless guanidine is added. On the other hand, when the buffer of *p*H 8 contains guanidine, all the cysteine-S of the wool is oxidized to cystine-S. These results suggest that some of the cysteine side-chains form thiazoline rings, masking the thiol groups, which become available for reaction only to certain reagents when other experimental conditions are favourable.

Our observations have an obvious bearing on the unmasking of thiol groups when sulphur-containing proteins are denatured[6] and the increase in free reactive thiol groups which occurs when guanidine hydrochloride is added to solutions of myosin[7] and albumin[8].

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Optical Projection as an Aid in Studying Plane Spiral Coiling of Small Fossil Lamellibranchs

THE use of optical projection in certain screw-gauges[1] and pivot-wear meters[2] suggested methods for examining the kind and degree of variation shown by plane spiral coiling in a group of small and well-preserved lamellibranchs (*Gryphœa incurva* (Sow.)) when researches upon these variations were extended[3] in this Department recently. Identical methods proved applicable to the study of small ammonites and other shells, whether fossil or recent, provided they were coiled in a plane spiral.

It was found that an accurate lantern-slide projector gave on a suitable screen a sharply defined silhouette of any lamellibranch up to 60 mm. in diameter when the fossil was held centrally in the lantern-slide carrier on a wax or 'Plasticine' pedestal, with the plane of coiling of the shell coincident with the focal plane of the projecting lens system.

For each specimen, by appropriately altering the distance between lantern and screen and sharply focusing its silhouette, a magnified but negligibly distorted image of constant diameter was obtained, the profile of which could be traced with a pencil on a sheet of opaque white paper, or tracing paper, attached to the screen.

Using opaque recording paper, comparisons between groups of these profiles were then facilitated by cutting along the profile outlines and superposing the 'cores' so separated from the sheets. With profiles drawn on tracing paper, the superposition of chosen profiles on an illuminated tracing desk rendered their comparison even simpler than with opaque paper.

Alternatively, the profile of a particular lamellibranch was directly compared with one of several