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Lanasol Dyes and Wool Fibres. Part II: Model Studies on the Mechanism of Dye Fixation in an Aqueous System

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ABSTRACT

In Part I of this study we investigated the mechanism of the fixation of Lanasol dyes to the various amino acid side chain sites within wool protein using model compounds in a mixed solvent system. As the dyeing of wool is generally carried out in an aqueous system at the boil it was felt that the model system would be more accurate if the compounds studied were water soluble. This paper reports on the synthesis of the sulfonated version of the model reactive dye compound used in Part I of this study and its subsequent reaction with model wool compounds. In addition, a model compound based on benzene sulfonic acid, and an actual commercial Lanasol dye were also reacted. As in the first part of this study the reaction products were isolated by chromatography and then characterized by proton and carbon-13 NMR, and electrospray mass spectroscopy. In general the results were consistent with those obtained from the study carried out in the acetone/water solvent system. It was confirmed that the dibromo form of the dye reactive group is only converted to the monobromo form in the presence of model wool compounds and that both forms react with model wool compounds to yield the same products. Amines reacted with the model dyes to form a product containing an aziridine ring—no evidence for the proposed reaction of this ring with a second nucleophilic wool site to form a cross-link between two protein chain segments could be detected. In the few cases where an aziridine ring structure was not formed, the products obtained were found to support a Michael addition (1,4-addition) reaction mechanism. Unlike the

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results found for the mixed solvent system it was found that the wool models for N-terminal groups did not react with the model dye compounds.

Keywords: wool fibres, Lanasol reactive dyes, mechanism of dye fixation.

INTRODUCTION

The investigation of the reactions of the α,β-dibromopropionamido and α-bromoacrylamido reactive groups with wool mimetics in acetone/water reported on in Part I of this work [1] provided a significant amount of new information about the products formed during wool dyeing with Lanasol type reactive dyes. The chemical groups used to mimic the wool protein reactive sites included primary amines, thiols, imidazole, phenolic and hydroxyl functional groups. The products of these reactions were isolated and fully characterised using ^{1}H and ^{13}C-NMR spectroscopy and mass spectrometry. A major draw back of the study however, was the poor aqueous solubility of the model dye compound used. This limited the study to reactions carried out in a mixed solvent system and thus relatively low temperatures. To more closely simulate the conditions used in the commercial dyeing process the model dye compound would have to be water soluble. For commercial dyes this is generally accomplished by the addition of sulfonate groups on either the chromophore or carrier group of the dye. In this study we report the synthesis of the sulfonated analogue of the model dye compound used in Part I and its subsequent reaction with the model wool compounds. The knowledge obtained from these model studies has enabled us to investigate the more complicated system involving an actual Lanasol dye. This work is also described.

EXPERIMENTAL

Chemicals and reagents

Thionyl chloride, α,β-dibromopropionic acid, sulphanilic acid, $NaHCO_3$, tetrabutylammonium bromide, methanol, 4-aminobenzophenone and anhydrous 1,2-dichlorobenzene, 98% sulphuric acid, sodium hydroxide and hydrochloric acid, anhydrous *N,N*-dimethylformamide, *N,N*-dimethylacetamide, 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU) were obtained from Sigma-Aldrich Chemical Company.

A sample of purified Lanasol Yellow 4G was received as a gift from Dr. Klaus Hannaman of Ciba Basel. This sample was found to have an extinction

coefficient at 395 nm over 40% greater than that of the commercial dye. All other chemicals and reagents used have been described elsewhere [1].

Instrumentation

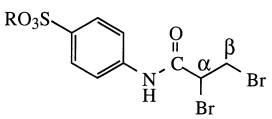
Thin layer chromatography (TLC) was carried out on Merck Kieselgel 60 F_{254} using an propan-2-ol/water system as specified. Ultraviolet light at 254 nm, ninhydrin and iodine vapour were used as detection methods. All other instrumentation used in this study has been presented elsewhere [1].

Preparation of model dyes

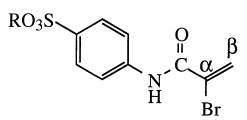
*α,β-Dibromopropionyl chloride (**1**)*
Thionyl chloride (5.8 ml, 55 mmol) was added to a 25 ml round bottomed flask under an atmosphere of nitrogen. A bubbler connected to a micro-condenser was then attached to the flask which was heated to 40°C with stirring. The bubbler was removed from the condenser and α,β-dibromopropionic acid (10.7 g, 46.1 mmol) was added in small aliquots. Each addition of the carboxylic acid was allowed to fully dissolve in the thionyl chloride prior to subsequent additions. Once all of the carboxylic acid had been added the bubbler was reattached and the solution was refluxed for 1 h at 90°C. Excess thionyl chloride was then distilled from the crude product which was then transferred to a nitrogen purged distillation apparatus via a gas tight syringe and heated to 210°C. The product was collected at 186–190°C (lit. bp = 191–193°C [2]). The resulting yield of α,β-dibromopropionyl chloride was 4.4 ml (83%). The FT–IR spectrum contained a strong absorbance assignable to the carbonyl stretch of the acid chloride at 1785 cm^{-1}, while the carboxylic acid carbonyl stretch at 1715 cm^{-1} was absent, thus confirming the formation of the acid chloride.

*4-(α,β-Dibromopropionamido)benzenesulphonic acid (**2**)*
Sulphanilic acid (4.40 g, 25.4 mmol) and deionised water (60 ml) were stirred at ambient temperature. $NaHCO_3$ (2.13 g, 25.4 mmol) was then slowly added to the suspension to form the water soluble sulphonate analogue of the acid. Dichloromethane (30 ml) and the phase transfer catalyst, tetrabutylammonium bromide (150 mg, 0.465 mmol), were added to a second flask and both flasks were cooled in an ice bath for 15 min. α,β-Dibromopropionyl chloride (3.5 ml, 31 mmol) was then added to the flask containing dichloromethane followed by the addition of the sulphanilic acid solution. This mixture was stirred at 0°C for 3 h and then overnight at room temperature. The organic layer was removed and the remaining solution was washed with dichloromethane (3×10 ml) to remove the residual phase transfer

catalyst. The aqueous solution was then evaporated *in vacuo* at 45°C and the crude product boiled for 5 min in methanol (50 ml). After gravity filtration of the hot solution to remove the small amount of insoluble material present, the flask was allowed to cool to room temperature. The white crystals that precipitated were collected by vacuum filtration, washed with methanol (3×10 ml) and dried under vacuum (0.5 mm Hg for 8 h). The resultant yield was 6.29 g (64%) and the product was found to decompose at 310°C. The structure and purity of the product was determined by ES–MS and NMR spectroscopy. The negative ion ES–MS of the product contained the expected parent ion at 386 m/z and incorporated the isotopic splitting characteristics of two bromine atoms. The ^{1}H-NMR spectrum of the product was free of resonances due to sulphanilic acid (doublets expected at 7.35 and 7.80 ppm in D_2O). The spectrum was recorded using D_2O since this solvent was used in later experiments, allowing a direct comparison of the spectra. Although the resonances due to the Hβ protons (3.72–3.93 ppm) were visible in this spectrum, the resonance due to Hα (4.68–4.78 ppm) was obscured by that of the solvent. The presence of the α proton was verified by recording the ^{1}H-NMR spectrum in DMSO-d_6. This spectrum also contained a singlet which could be assigned to the sulphonic acid proton (10.91 ppm) which was not observed in D_2O due to deuterium-hydrogen exchange. The amide proton was not observed in the spectrum recorded in DMSO-d_6 even though evidence for the formation of an amide bond was provided by the (-) ES–MS parent ion of the product. No evidence of any α,β-dibromopropionic acid (formed from the hydrolysis of excess α,β-dibromopropionyl chloride) was observed by NMR. ^{1}H-NMR (D_2O) δ 3.72–3.93 (m, 2H, Hβ), 4.68–4.78 (m, 1H, Hα), 7.52 (d, 2H, ArH), 7.71 (d, 2H, ArH).



2 R = H/Na



3 R = H/Na

*4-(α-Bromoacrylamido)benzenesulphonic acid (**3**)*

4-(α,β-Dibromopropionamido)benzenesulphonic acid (15 mg, 39 mmol) was dissolved in D_2O (0.8 ml) in an NMR tube. $NaHCO_3$ (8 mg, 95 mmol) was then slowly added to the tube with stirring, after which the tube was heated to 80°C for 15 min on a water bath. A large molar excess of $NaHCO_3$ was required since the dehydrobromination process had to compete with the conversion of the sulphonic acid group to a sulphonate group. A ^{1}H-NMR spectrum of the reaction solution was recorded which showed that the desired product had formed. The spectrum of the alkaline solution contained

no dibromopropionyl resonances at 3.72–3.93 ppm and 4.68–4.78 ppm, while new resonances due to non-equivalent acryl protons were observed at 6.23 and 6.70 ppm. Confirmation was also gained from the negative ion ES–MS of the alkaline solution, which contained the expected parent ion for the product (**3**) at 304 and 306 m/z (splitting due to one bromine atom) and no parent ion corresponding to starting material (triplet expected to be centred at 386 m/z).

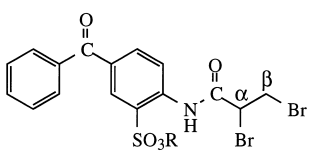
*4-Aminobenzophenone-3-sulphonic acid (**4**)*

4-Aminobenzophenone (10.00 g, 50.7 mmol) was suspended in anhydrous 1,2-dichlorobenzene (40 ml) in an oven dried 100 ml round bottom flask containing a reflux condenser. The mixture was heated to 100°C followed by the slow addition of 98% sulphuric acid (3.24 ml, 60.8 mmol) through the condenser with vigorous stirring. The mixture was then refluxed at 180°C for 6 h. After rearranging the apparatus, the temperature of the flask was raised to 200°C allowing the 1,2-dichlorobenzene to be distilled from the reaction mixture. A solution of sodium hydroxide (2.90 g, 72.5 mmol) in water (50 ml) was added to the crude product, as well as a spatula of activated charcoal, and the mixture was warmed to 60°C with stirring. After cooling to room temperature, the unreacted 4-aminobenzophenone that precipitated and the charcoal, were collected by vacuum filtration. The liquor was placed in a 100 ml separating funnel and the residual 1,2-dichlorobenzene was removed after standing for 30 min. The aqueous solution was then placed in a 100 ml round bottom flask and acidified with concentrated hydrochloric acid to precipitate the product. After collecting the product by vacuum filtration the yellow crystals were washed with 0.5 M HCl (3×10 ml) and dried under vacuum (0.5 mm Hg) for 8 h resulting in a yield of 10.68 g (76%). The melting point of the product was found to be 306–308°C. The negative ion ES–MS contained the expected parent ion at 276 m/z. The following ^{1}H and ^{13}C-NMR spectral assignments observed for the product were consistent with those calculated for 4-aminobenzophenone-3-sulphonic acid: ^{1}H-NMR (DMSO-d_6): δ 6.77 (d, 1H), 7.47-7.63 (m, 6H, ArH), 7.93 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 116.9 (t), 125.4 (q), 125.6 (q), 128.8 (t), 129.3 (t), 131.0 (t), 132.0 (t), 132.7 (t), 138.9 (q), 147.1 (q), 194.1 (q).

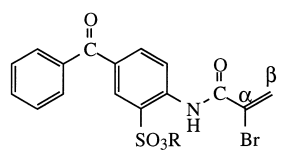
*4-(α,β-Dibromopropionamido)benzophenone-3-sulphonic acid (**5**)/4-(α-bromoacrylamido)benzophenone-3-sulphonic acid (**6**) mixture*

4-Aminobenzophenone-3-sulphonic acid (**4**) (4.00 g, 14.4 mmol) was dissolved in deionised water (50 ml) and converted to the corresponding sodium sulphonate by the slow addition of $NaHCO_3$, and stirring at room temperature until the pH of the solution was stable at 7. Water was then evaporated using a rotavap at 45°C and the yellow powder remaining was dried under

vacuum (0.5 mm Hg) for 8 h. This conversion was necessary to improve the solubility of the sulphonated aminobenzophenone compound in the solvents used for the preparation of the desired model compounds. The dry powder was added to a dry 100 ml round bottomed flask containing a magnetic stirrer bar and then capped with a rubber septum. Dry *N,N*-dimethylacetamide (DMA, 30 ml) was added to the flask through the septum via a gas tight syringe. Once the powder had dissolved, the flask was placed in an ice bath and the solution stirred for 15 min. α,β-Dibromopropionyl chloride (2.0 ml, 17.3 mmol) was then added dropwise to the solution via a 5 ml gas tight syringe and the reaction mixture was stirred vigorously for 30 min. During the addition of the acid chloride the formation of a precipitate was observed. Pressure build up in the flask was released by periodically inserting a syringe needle connected to a paraffin oil filled bubbler via a rubber tube through the septum. The solution was stirred at 0°C for 3 h and then overnight at room temperature. DMA was evaporated and the crude product was dissolved in deionised water (50 ml) and transferred to a 250 ml separating funnel. Additional deionised water (50 ml) was added to the funnel and the pH of the solution was raised to 8 with $NaHCO_3$. The solution was then washed with chloroform (5×15 ml) to remove the residual DMA from the aqueous phase, and the product mixture was extracted into ethyl acetate (5×15 ml). The product was kept under alkaline conditions for a minimal time to avoid hydrolysis of the amide bond. Acidification of the ethyl acetate phase (conc. HCl) followed by evaporation of the solvent afforded the crude product. Thin layer chromatography revealed the presence of a mixture of compounds **5** and **6**.



5 R = H/Na



6 R = H/Na

The product mixture was chromatographed on silica gel using ethylacetate/acetone/water(80/30/7 v/v), but satisfactory separation of compounds **5** and **6** was not achieved. The fractions containing **5** and **6** were combined and the solvent was removed under reduced pressure. The remaining yellow powder was vacuum dried (0.5 mm Hg) for 16 h resulting in a yield of 4.13 g having a melting point of 267–270°C. The structure and purity of the product was analysed by ES–MS and ^{1}H-NMR spectroscopy. The negative ion ES–MS of the product contained two parent peaks due to **5** (488, 490 and 492 m/z) and **6** (408 and 410 m/z). The splitting of these peaks was expected due to the presence of two bromine atoms in the α,β-dibromopropionamido analogue and one bromine atom in α-bromoacrylamido analogue. The ratio of **5** to **6** was

calculated to be approximately 3:2 based on the relative abundance of the parent ion peaks. This ratio was confirmed from the ^{1}H-NMR spectrum of the product, based on the comparison of the peak area corresponding to Hβ for the α,β-dibromopropionylamido and α-bromoacrylamido forms. Apart from compounds **5** and **6**, the product also contained approximately 10% α-bromoacrylic acid which was produced by hydrolysis of the excess α,β-dibromopropionyl chloride and/or cleavage of amide bonds during the extractions under alkaline conditions. It was not possible to reduce the molar ratio of this contaminant by further extractions or column chromatography, as it co-eluted with the product. ^{1}H-NMR (D_2O) δ 3.80 (d, 2H, Hβ), 4.82 (t, 1H, Hα), 6.19 (s, 1H, Hβ*), 6.80 (s, 1H, Hβ*), 7.33-8.49 (m, ArH and ArH*) (* = α-bromoacrylamido form). It was not possible to record a ^{13}C-NMR spectrum of the product of reasonable S/N ratio since the high concentrations required to attain a reasonable acquisition time resulted in rapid polymerisation.

Reactions of the model dyes with ethylamine

*Reaction of 4-(α,β-dibromopropionamido)benzenesulphonic acid (**2**) with ethylamine*

α,β-Dibromopropionamido)benzenesulphonic acid (15 mg, 39 mmol) was dissolved in D_2O (0.8 ml) in an NMR tube. A 70% aqueous solution of ethylamine (7.5 ml, 116 mmol) was added to the NMR tube via a syringe and the pH of the solution was adjusted to 5 with 1 M HCl solution prepared from concentrated HCl and D_2O. The tube was placed in a 400 ml beaker of water and maintained at 100°C for 90 min with magnetic stirring. ^{1}H-NMR (D_2O) δ 1.05 (t, 3H, NCH_2CH_3), 1.17* (t, 6H, $CH_3CH_2NH_2$), 1.74 (s, 1H, Hβ), 2.06 (s, 1H, Hβ) 2.35 (q, 2H, NCH_2CH_3), 3.93* (q, 4H, $CH_3CH_2NH_2$), 7.52 (d, 2H, ArH), 7.69 (d, 2H, ArH) (* = unreacted ethylamine).

*Reaction of 4-(α-bromoacrylamido)benzenesulphonic acid (**3**) with ethyl amine*

4-(α-Bromoacrylamido)benzenesulphonic acid (**3**) was prepared from **2** (15 mg, 39 mmol) in D_2O in an NMR tube according to the method used in Part I of this study. The mixture was then reacted under the same conditions described for the reaction of compound **2** with ethylamine (above). A ^{1}H-NMR was recorded which proved to be identical to the one described above.

Reactions of the model dyes with wool mimetics

The reactions of the model dye mixture (**5** + **6**) with model compounds were performed in a similar manner to the reactions of Part I, except that the

reactions were performed in deionised water (10 ml) in a 25 ml round bottom flask at 100°C for 90 min. If the pH of the solution was to be raised in the presence of the model dye mixture (**5** + **6**) then saturated $NaHCO_3$ solution was used instead of solid $NaHCO_3$. This change was made in order to reduce the possibility of hydrolysing the amide bonds of the model dyes.

The mass of the model dye mixture (**5** + **6**) used for each reaction with the model wool compounds was 300 mg. This product contained 90% of model dye compound, which is equivalent to 0.38 mmol of **5** and 0.25 mmol of **6**. It was felt that the 10% presence of α-bromoacrylic acid would not have an effect on the outcomes of the reactions with the model wool compounds. The reaction mixtures were analysed by TLC using propan-2-ol/water (4:1 to 19:1 v/v) as the mobile phase. If a reaction product was observed, the entire reaction mixture was then separated by column chromatography on silica gel. After evaporation of the mobile phase the product was vacuum dried (0.5 mm Hg) for 8 h.

*Reaction of the model dye mixture (**5** + **6**) with* Nα-*acetyl*-L-*lysine*-N-*methylamide*

The model dye mixture (**5** + **6**) was reacted with *N*α-acetyl-L-lysine-*N*-methylamide (2.03 g, 10.1 mmol), resulting in a dye mixture to amino acid molar ratio of 1:16. TLC results revealed that the reaction mixture contained a component at R_f 0.40 which could only be assigned to a new product, while the starting materials were no longer observed. Like their non-sulphonated analogues, compounds **5** and **6** were also observed to have virtually the same retention on silica gel. Column chromatography of the entire reaction mixture resulted in 311 mg of a pale yellow powder (93% yield) that decomposed at 315°C. ^{1}H-NMR (D_2O) δ 1.24-1.51 (m, 4H, 2×lysCH_2), 1.51–1.68 (m, 2H, lysCH_2), 1.74 (s, 3H, $COCH_3$), 1.82 (s, 1H, Hβ), 1.97 (s, 1H, Hβ), 2.03 (m, 2H, lysCH_2), 2.19 (br s, 1H, Hα), 2.52 (s, 3H, N-CH_3), 3.99 (t, 1H, lysCH), 7.37-8.05 (m, 8H, ArH); ^{13}C-NMR (D_2O) δ 21.6, 22.9, 25.8, 28.2, 30.8, 34.5, 39.5, 54.3, 58.1, 121.5, 128.5, 129.3, 129.9, 131.8, 132.1, 133.4, 134.0, 136.0, 137.7, 171.6, 174.2, 174.8, 197.4.

To test the effect of the ammonia after-treatment used in commercial wool dyeing, the reaction with *N*α-acetyl-L-lysine-*N*-methylamide was repeated, but after a reaction time of 90 min, the temperature was reduced to 80°C and the pH of the solution adjusted to 8 with 25% ammonia solution. The solution was allowed to stir at this temperature for a further 15 min, after which a sample of the reaction mixture was separated by TLC. The results from the TLC were identical to those obtained without the after-treatment. The results of column chromatography were also very similar; 95% yield and decomposition at 315°C. The ^{1}H- and ^{13}C-NMR spectra were identical to those described above.

*Reaction of the model dye mixture (**5**+**6**) with* Nα-*acetyl-*L-*histidine*
The model dye mixture (**5**+**6**) was reacted with *N*α-acetyl-L-histidine (585 mg, 2.96 mmol), resulting in a dye mixture to amino acid molar ratio of 1:4.7. TLC results showed that the reaction mixture contained a component at R_f 0.71 due to a new product and there was no evidence of starting material. Column chromatography was used to isolate this new product as a pale yellow powder (353 mg, 92% yield) with a melting point in the range of 329-331°C. ^{1}H-NMR (D_2O) δ 1.68–1.93 (m, 5H, $COCH_3$, Hβa and b), 2.64–3.02 (dd, 2H, hisCH_2), 4.18-4.56 (m, 2H, Hα and hisCH), 6.90 (3, 1H, hisCH), 7.30-8.03, 9H, ArH and hisCH); ^{13}C-NMR (D_2O) δ 21.9, 26.2, 29.7, 47.2, 55.0, 118.2, 121.2, 128.5, 129.3, 129.9, 132.1, 132.4, 133.4, 134.0, 135.9, 136.0, 137.1, 138.0, 166.5, 171.5, 173.3, 197.2.

*Reaction of model dye mixture (**5**+**6**) with* N-*acetyl-*L-*cysteine*
The model dye mixture (**5**+**6**) was reacted with *N*-acetyl-L-cysteine (268 mg, 1.64 mmol), resulting in a dye mixture to amino acid molar ratio of 1:2.6. TLC of the reaction mixture revealed that no starting material was left. A new spot, identified by UV light, co-eluted with the excess unreacted *N*-acetyl-L-cysteine (detected by iodine vapour). Modification of the mobile phase solvent ratios between the range 4:1 and 19:1 (v/v) did not improve the results of the separation.

An attempt to separate the reaction components was made by changing the polarity of the excess thiol by reacting it with 2,4-dinitrofluorobenzene (DNFB). DNFB (0.127 ml, 1.01 mmol) was added to the model dye/*N*-acetyl-L-cysteine reaction mixture, the pH of this solution was raised to 8 with saturated $NaHCO_3$ solution and the contents allowed to stir for 30 min at room temperature. A sample of the reaction mixture was then separated by TLC using propan-2-ol/water (9:1 v/v) as the mobile phase. The TLC of the reaction mixture contained a component at R_f 0.44 due to a new product and a component at R_f 0.25 due to the dinitrophenylated amino acid, *S*-dinitrophenyl-*N*-acetyl-L-cysteine. Spots were not observed for free *N*-acetyl-L-cysteine or DNFB, which suggests that these compounds reacted completely. The entire reaction mixture was then separated by column chromatography using propan-2-ol/water (9:1) as the mobile phase. The resulting pale yellow powder weighed 281 mg (90% yield) and melted at 322–324°C. ^{1}H-NMR (D_2O) δ 1.75–1.89 (m, 5H, $COCH_3$, Hβa and b), 2.70-3.05 (m, 4H, Hα and cysCH_2), 4.21 (t, 1H, cysCH), 7.41-8.10 (m, 8H, ArH); ^{13}C-NMR (D_2O) δ 21.8, 33.0, 33.8, 34.6, 54.5, 122.3, 128.6, 129.5, 130.1, 132.3, 132.7, 133.5, 134.2, 136.2, 137.6, 173.6, 176.4, 176.8, 198.3.

*Reaction of the model dye mixture (**5**+**6**) with* N-*acetyl-*L-*serine*
The model dye mixture (**5**+**6**) was reacted with *N*-acetyl-L-serine (5.29 g, 36.0 mmol) resulting in a dye mixture to amino acid molar ratio of 1:57. Due

to the large mass of amino acid used in this experiment the reaction was performed in deionised water (20 ml) in a 50 ml round bottom flask. Both the model dye mixture and *N*-acetyl-L-serine could be detected by TLC. No spots were observed that were indicative of new product formation.

*Reaction of the model dye mixture (**5**+**6**) with glycine*
The model dye mixture (**5**+**6**) was reacted with glycine (62 mg, 0.82 mmol), resulting in a dye mixture to model compound molar ratio of 1:1.3. The TLC analysis of the reaction mixture revealed only starting materials (**5**+**6** and glycine). No spots were observed that indicated the presence of a reaction product. Changes to the mobile phase solvent ratios ranging from 4:1 to 19:1 v/v did not aid in revealing any new components that may have been co-eluting with the starting materials.

Reactions of a commercial Lanasol dye with wool mimetics

The reactions involving purified Lanasol Yellow 4G (350 mg, 0.50 mmol) with model wool compounds were performed under identical conditions and molar ratios to those involving the model dye mixture (**5**+**6**). Components were detected on the TLC plates using both natural light and iodine vapours.

Reaction of Lanasol Yellow 4G with Nα-*acetyl*-L-*lysine*-N-*methylamide*
The purified Lanasol dye was reacted with *Nα*-acetyl-L-lysine-*N*-methyl-amide (1.61 g, 8.02 mmol), resulting in a dye to amino acid molar ratio of 1:16. The results of the TLC revealed that the reaction mixture contained a single component at R_f 0.62, which was probably due to a new product as the Lanasol dye component was not observed. Column chromatography separation of the entire reaction mixture resulted in the isolation of 390 mg of product (95% yield) which decomposed at 320°C. ^{1}H-NMR (D_2O) δ 1.14-1.65 (m, 6H, 3×lysCH_2), 1.79 (s, 3H, $COCH_3$), 1.84 (s, 1H, Hβ), 1.93 (s, 3H, CH_3), 2.06 (br s, 3H, Hβ and lysCH_2), 2.25 (br s, 1H, Hα), 2.54 (s, 3H, N-CH_3), 3.98 (br s, 1H, lysCH), 7.33-8.04 (m, 5H, ArH); ^{13}C-NMR (D_2O) δ 6.4, 17.1, 17.8, 21.3, 23.6, 26.4, 29.3, 34.6, 49.7, 54.3, 101.9, 112.0, 121.9, 122.9, 124.0, 124.3, 125.4, 125.9, 126.3, 131.5, 133.4, 136.2, 146.9, 151.3, 165.5, 169.7, 170.2.

Reaction of Lanasol Yellow 4G with Nα-*acetyl*-L-*histidine*
The purified Lanasol dye was reacted with *Nα*-acetyl-L-histidine (464 mg, 2.35 mmol), resulting in a Lanasol dye to amino acid molar ratio of 1:4.7. The results revealed that the reaction mixture contained a single component at R_f 0.55. Column chromatography was used to isolate 422 mg of a single component (94% yield) which decomposed at 335°C. ^{1}H-NMR (D_2O) δ 1.76

(s, 3H, $COCH_3$), 1.81 (s, 2H, Hβ), 1.96 (s, 3H, CH_3), 2.60-3.12 (m, 2H, hisCH_2), 4.13–4.44 (m, 2H, Hα and hisCH), 6.85 (s, 1H, hisCH), 7.12-8.02 (m, 6H, ArH and hisCH); ^{13}C-NMR (D_2O) δ 13.3, 24.3, 28.6, 32.3, 49.2, 57.4, 108.4, 118.3, 120.4, 127.9, 128.7, 129.6, 130.9, 132.1, 132.6, 133.5, 138.4, 139.8, 140.4, 140.7, 142.7, 143.1, 153.6, 157.9, 168.9, 175.8, 180.9.

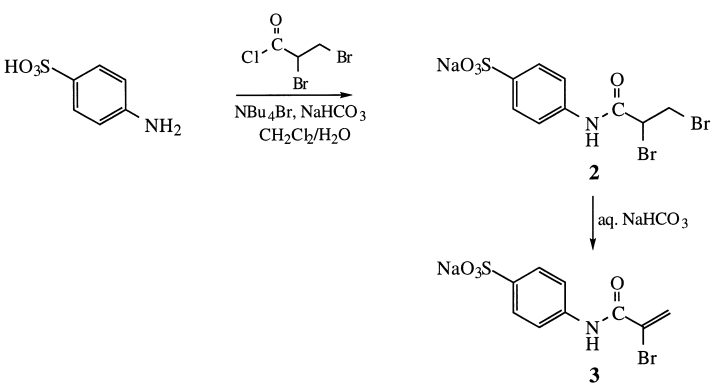
Reaction of Lanasol Yellow 4G with N-*acetyl*-L-*cysteine*

The purified Lanasol dye was reacted with *N*-acetyl-L-cysteine (213 mg, 1.30 mmol), resulting in a Lanasol dye to amino acid molar ratio of 1:2.6. Attempts to separate the reaction mixture by TLC using a 4:1 (v/v) propan-2-ol/water mobile phase were not successful. A spot due to the Lanasol dye was not observed, but a new spot was observed (detectable by natural light) which co-eluted with the excess unreacted *N*-acetyl-L-cysteine. An unsuccessful attempt was made to separate these two components by varying the solvent ratio between 4:1 and 19:1.

RESULTS AND DISCUSSION

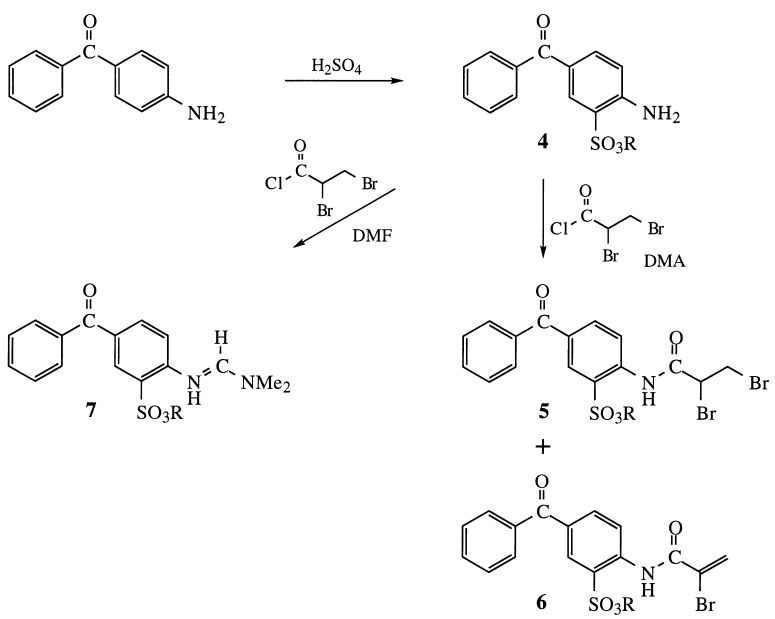
Model dyes

Model dye compounds 4-(α,β-dibromopropionamido)benzenesulphonic acid (**2**) and 4-(α-bromoacrylamido)benzenesulphonic acid (**3**) were prepared from commercially available sulphanilic acid using the straightforward approach outlined in Scheme 1. Compound **2** was prepared by treating sulphanilic acid with α,β-dibromopropionyl chloride under phase transfer conditions. Compound **2** was then converted to **3** via a base (sodium bicarbonate) induced elimination reaction.



Scheme 1.

Sulphonated analogs of the model dyes used in Part I of this study were prepared to enable comparative studies to be performed in an exclusively aqueous medium. A mixture of 4-(α,β-dibromopropionamido)benzophenone-3-sulphonic acid (**5**) and 4-(α-bromoacrylamido)benzophenone-3-sulphonic acid (**6**) was prepared using the synthesis shown in Scheme 2. The first step in this synthesis was the sulphonation of 4-aminobenzophenone. Common procedures for sulponation of aromatic systems [3, 4] proved to be problematic in this case. The desired product (**4**) was eventually obtained in 78% yield by treating 4-aminobenzophenone with concentrated sulphuric acid in hot 1,2-dichlorobenzene. The synthesis of 4-(α-bromoacrylamido)-benzophenone-3-sulphonic acid (**5**) was initially attempted by treating 4-aminobenzophenone-3-sulphonic acid with α,β-dibromopropionyl chloride in dry DMF. An extensive investigation into the structure of the product using ^{1}H and ^{13}C-NMR, mass spectroscopy and elemental analysis revealed that the amine had reacted with the solvent, DMF, to produce 4-(*N,N*-dimethylimido)benzophenone-3-sulphonic acid (**7**). To prevent this undesired reaction dry *N,N*-dimethylacetamide (DMA) was used as the solvent in place of DMF. The carbonyl group of DMA was expected to be less reactive than DMF towards nucleophilic addition due to the steric and electronic effects of the additional methyl group [5]. This proved to be the case as no reaction with the solvent was observed when 4-aminobenzophenone-3-sulphonic acid



Scheme 2. R = H/Na.

was treated with α,β-dibromopropionyl chloride in dry DMA. This reaction afforded a mixture of the **5** and **6** in a 3:2 molar ratio (Scheme 2). Formation of compound **6** formed via spontaneous elimination of HBr from **5** could not be avoided by modifying the reaction conditions.

Attempts were made to convert the α,β-dibromopropionamido component of the product into α-bromoacrylamido so that only one reactive group was present. This is normally achieved with an alkali (e.g. $NaHCO_3$ or NaOH), however the sulphonated dye was found to be unstable in the presence of these nucleophilic bases. The dye was treated with the sterically hindered, weakly nucleophilic base, 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU), which dehydrobrominated **5** without cleaving the amide bond. Unfortunately DBU also caused compound **6** to precipitate as a polymer. Therefore, the sulphonated model dye was employed as a mixture of the α,β-dibromopropionamido and α-bromoacrylamido forms for reaction with model wool compounds. A number of commercial Lanasol dyes are known to exist as a mixture of these two reactive forms [6].

The effect of water on 4-(α,β-dibromopropionamido)benzenesulphonic acid (2)

The effect of water on the α,β-dibromopropionamide group was investigated to determine whether the α-bromoacrylamide group would be formed by dehydrobromination. 4-(α,β-Dibromopropionamido)benzenesulphonic acid was dissolved in D_2O and subjected to conditions used for commercial dyeing operations (100°C, pH 5, 90 min). A ^{1}H-NMR spectrum of this solution was then recorded and the amount of HBr removed from the sample was calculated from the ratio of the bromoacryl peak area to the dibromopropionyl peak area. It was found that after boiling for 90 min only 4% of the starting material was converted to 4-(α-bromoacrylamido)benzenesulphonic acid (**3**) which shows the amount of dehydrobromination that occurred under these conditions was insignificant.

Reaction of model dyes with wool mimetics

4-(α,β-Dibromopropionamido)benzenesulphonic acid (**2**) and 4-(α-bromoacrylamido) benzenesulphonic acid (**3**) were used as model Lanasol dyes for *insitu* ^{1}H-NMR spectroscopic studies as their preparation in a relatively pure form was uncomplicated. The reaction of both forms of the Lanasol reactive group with wool mimetic was then tested separately. 4-(α,β-Dibromopropionamido)benzophenone-3-sulphonic acid (**5**) and 4-(α-bromoacrylamido) benzophenone-3-sulphonic acid (**6**), sulphonated analogues of the model dyes used in the acetone/water work of Part I of this work, were prepared and used as a mixture since separation of the components proved difficult.

The mixture was reacted with wool amino acid mimetics and the products were isolated and characterised by the same spectroscopic techniques used for the non-sulphonated derivatives. The information acquired from the studies in acetone/water aided in the determination of the structure of the products obtained from the reactions in aqueous media.

Comparison of the reactivity of the α,β-*dibromopropionamide and* α-*bromoacrylamide moieties*

Since the α,β-dibromopropionamide group was found to be stable under commercial dyeing conditions in water, it was necessary to examine whether it and the α-bromoacrylamido group would form the same products when reacted with a wool functional group. The negative ion ES–MS of the reaction mixtures of both 4-(α,β-dibromopropionamido)benzenesulphonic acid (**2**) with ethylamine and 4-(α-bromoacrylamido)benzenesulphonic acid (**3**) with ethylamine were found to be identical, both spectra containing a parent ion at 270 m/z. This ion is consistent with either a product containing an ethylamine molecule that had substituted the α-bromine of **3** or a product containing an aziridine ring. Parent ions were not observed for a product containing an ethylamine molecule that had reacted across the double bond of **3** (expected at 350 m/z) or for a product containing two molecules of ethylamine (expected at 314 m/z).

The ^{1}H-NMR spectra recorded from the reaction mixtures of **2** with ethylamine and **3** with ethylamine were also found to be identical. The spectra of the solutions did not contain resonances in the region expected for acryl protons which eliminated the α-bromine substituted structure. Resonances due to aziridine protons were assigned to Hβa and b (2.06 and 1.74 ppm). The resonance due to Hα was not observed in this spectrum, probably due to this proton being exchanged by deuterium during the 90 min long exposure to D_2O. The process of subjecting aziridine compounds to a deuterated solvent to assist in the assignment of the aziridine methyne group has been previously reported [7].

*Reaction of the model dye mixture (**5**+**6**) with* Nα-*acetyl*-L-*lysine*-N-*methylamide*

The purified reaction product had a negative ion ES–MS parent ion at 529 m/z. This ion was consistent with either a product containing an *N*α-acetyl-L-lysine-*N*-methylamide molecule that had substituted the α-bromine of **6** or with a product that contained an aziridine ring. Parent ions were not observed for a product containing an *N*α-acetyl-L-lysine-*N*-methylamide molecule that had reacted across the double bond of dehydrobrominated **5** (expected at 610 m/z) or for a product containing two molecules of *N*α-acetyl-L-lysine-*N*-methylamide (expected at 731 m/z).

It was possible to determine the structure of the product isolated from the reaction of the model dye mixture (**5** + **6**) with *N*α-acetyl-L-lysine-*N*-methylamide by NMR spectroscopy. The ^{1}H-NMR spectrum of the product was free of resonances in the region expected for acryl protons, which eliminated the α-bromine substituted structure. Resonances due to aziridine protons were assigned to Hα (2.19 ppm) and Hβ (1.97 and 1.82 ppm). Confirmation of this structure was obtained from the ^{13}C-NMR spectrum of the isolated product. Resonances were not observed for acryl carbons, with all resonances in this region being assigned to aromatic carbons due to the sulphonated benzophenone moiety of the product. Resonances due to the α and β-carbons of the aziridine ring were observed at 39.5 and 34.5 ppm, respectively, and are consistent with those observed for the 4-(α,β-dibromopropionamido) benzophenone/*N*α-acetyl-L-lysine-*N*-methylamide product [1].

The product isolated from the reaction mixture subjected to the ammonia aftertreatment was also characterised by ES–MS, ^{1}H and ^{13}C-NMR spectroscopy. These analyses showed that the ammonia treatment had no affect on the structure of the product. A product containing two wool reactive groups covalently bound to one model dye reactive group was not observed, which indicates that such an aftertreatment would not produce cross-links between the dye and wool protein. The mechanism of the reaction of the model dye mixture with *N*α-acetyl-L-lysine-*N*-methylamide would be analogous to that of the other primary amines considered in this investigation.

*Reaction of the model dye mixture (**5** + **6**) with* Nα*-acetyl-L-histidine*

The reaction product had a negative ion ES–MS parent ion which displayed bromine isotopic peaks at 605 and 607 *m*/*z*. A parent ion at this position was only expected for a product that contained an *N*α-acetyl-L-histidine molecule that had either substituted the β-bromine atom of **5** or reacted at the β-carbon of **6** via a Michael addition. Parent ions were not observed for a product containing an *N*α-acetyl-L-histidine molecule that had substituted the α-bromine of **6** (expected at 525 *m*/*z*) or for a product containing two molecules of *N*α-acetyl-L-histidine (expected at 723 *m*/*z*).

The structure of the product was confirmed by NMR spectroscopy. As anticipated, the ^{1}H-NMR spectrum of isolated product contained no acryl resonances, while the resonance at 6.90 ppm was assigned to a methyne imidazole proton. The region from 4.18 to 4.56 ppm integrated as two protons, which were assigned to the methyne resonance of the amino acid backbone of covalently bound histidine and to the resonance due to CH-Br. This latter assignment compared well with the CH-Br resonance (α-position) of **5** observed at 4.82 ppm. These conclusions were supported by the ^{13}C-NMR spectrum. Acryl carbons were not observed in the expected region of

the ^{13}C-NMR spectrum of the product. The resonances in this region were assigned to the sulphonated benzophenone aromatic carbons and imidazole carbons of the product. The resonance at 47.2 ppm was more characteristic of a CH-Br carbon than of an aziridine methyne carbon, as it corresponded well to the CH-Br resonance (α-position) observed in the ^{13}C-NMR spectrum of 4-(α,β-dibromopropionamido)benzophenone at 46.4 ppm [1]. The presence of an aziridine ring within the structure of the product was discounted, since the difference between the α and β-carbon resonances was too large (17.5 ppm) based on the amine model wool compound reactions previously studied in this investigation and the work of Yazhen *et al.* [7] (3–8 ppm). It was concluded that the reaction of the model dye mixture with *N*α-acetyl-L-histidine resulted in a product that contained the amino acid bound via an imidazole nitrogen to the β-carbon of the model dye, while no other products were observed. The mechanism of the reaction of **5** with *N*α-acetyl-L-histidine would involve nucleophilic substitution of the β-bromine, while the corresponding reaction mechanism for **6** would involve a Michael addition of the imidazole amine at the β-carbon.

*Reaction of the model dye mixture (**5**+**6**) with* N-*acetyl*-L-*cysteine*

The negative ion ES–MS of the reaction mixture contained a parent ion at 493 m/z which is expected for a product containing an *N*-acetyl-L-cysteine molecule attached to the β-carbon of the model dye while the α-carbon was reduced with the removal of the bromine. Parent ions were not observed for a product containing an *N*-acetyl-L-cysteine molecule that had reacted across the double bond of **6** without loss of the α-bromine or reacted via nucleophilic substitution of the β-bromine of **5** (the same product occurs from either situation and is expected at 573 m/z). Also, a parent ion was not observed for a product containing two molecules of *N*-acetyl-L-cysteine (expected at 655 m/z). Of the reactants, a molecular ion for *N*-acetyl-L-cysteine was detected at 162 m/z while those for **5** (expected at 489 m/z) and **6** (expected at 408 m/z) were not observed. The presence or absence of these particular reactants in the reaction solution determined by ES–MS are consistent with the TLC results.

The 2,4-dinitrofluorobenzene treated reaction mixture was separated by column chromatography and the isolated product analysed by ES mass spectrometry and NMR spectroscopy. The negative ion ES–MS parent ion of the product was unchanged (493 m/z) to that prior to treatment with 2,4-dinitrofluorobenzene, indicating that the treatment did not interfere with the product. Verification of the TLC results showing that the free *N*-acetyl-L-cysteine in solution had reacted completely with 2,4-dinitrofluorobenzene was provided from this mass spectrum, since a molecular ion for the free amino acid (expected at 162 m/z) was not observed. The ^{1}H-NMR spectrum of the

product contained the expected three methylene resonances at 1.75–1.89 ppm and 2.70–3.05 ppm. Resonances due to methyne-bromine, methylene-bromine and acryl groups were absent from the spectrum, which provides supporting evidence for the structure indicated by mass spectrometry. The same conclusions about the structure of the product were made using ^{13}C-NMR spectroscopy. The new methylene groups were observed at around 34 ppm and verified from the corresponding DEPT-NMR spectrum.

It was concluded that the reaction of the model dye mixture with *N*-acetyl-L-cysteine resulted in a product that contained the amino acid bound via its thiol group to the β-carbon of the model dye, while the α-carbon was reduced with the removal of the bromine. The reaction mechanism presumably involved nucleophilic substitution (in the case of compound **5**) or Michael addition (for compound **6**) followed by reduction at the α-carbon.

*Reaction of the model dye mixture (**5**+**6**) with* N-*acetyl-serine*

No reaction between the model dye mixture and *N*-acetyl-L-serine was observed.

*Reaction of the model dye mixture (**5**+**6**) with glycine*

It was observed that, in the acetone/water solvent system, covalent bonding occurred between 4-(α,β-dibromopropionamido)benzophenone and compounds that simulated the *N*-terminal groups of wool protein [1]. It was expected that the same reactions would occur using conditions that resembled the commercial dyeing process more closely. The model wool compounds used in the acetone/water studies were *N*-carbobenzoxy-L-lysine and *S*-carboxymethyl-L-cysteine, which both contain α-amine groups free for reaction with the model dye, while the side chains were rendered unreactive by the presence of a protecting group. However, it was not possible to use these wool amino acid mimetics in this investigation, since they are insoluble in water under the conditions used in this investigation. A commonly used water soluble alternative is glycine which contains a free α-amino group and no side chain.

As mentioned above, the TLC analysis of the model dye/glycine reaction indicated that there were no products formed. Likewise, the ES–MS of the reaction solution did not contain any molecular ions indicative of product formation. Thus, it was concluded that unlike the reaction between 4-(α,β-dibromopropionamido)benzophenone and the *N*-terminal models in acetone/water [1], no reaction occured between the model dye mixture (**5**+**6**) and glycine. A similar observation was reported by Virnik and Chekalin [8], who reacted a dichlorotriazine dye with glycine at pH 5–6. These workers found that the reaction was more rapid in aqueous alcohol or dioxan than in water,

which indicated that the uncharged form of glycine reacted more readily than the zwitterion.

Reactions of a commercial Lanasol dye with wool mimetics

The more ideal study of the mechanism of fixation of the Lanasol reactive groups to wool would involve an actual commercial Lanasol dye instead of a model dye. To this end, we have investigated the reaction of Lanasol Yellow 4G with *N*α-acetyl-L-lysine-*N*-methylamide, *N*α-acetyl-L-cysteine and *N*α-acetyl-L-histidine under commercial dyeing conditions. It should be noted that this dye has two sulphonate groups and therefore a two minus charge in solution; thus the negative ion ES–MS parent ion of the products containing this dye will be equal to half of the molecular mass of the doubly charged anion.

*Reaction of Lanasol Yellow 4G with N*α*-acetyl-*L*-lysine-*N*-methylamide*

The purified product was found to have a ES–MS parent ion at 386 m/z. This ion was consistent with either a product containing an *N*α-acetyl-L-lysine-*N*-methylamide molecule that had substituted the α-bromine of the Lanasol dye or of a product that contained an aziridine ring. These possibilities are analogous to those for the reaction of 4-(α,β-dibromopropionamido)benzophenone or the model dye mixture (**5** + **6**) with *N*α-acetyl-L-lysine-*N*-methylamide. Parent ions were not observed for a product containing an *N*α-acetyl-L-lysine-*N*-methylamide molecule that had reacted across the double bond of the dye reactive group (expected at 426 m/z) or of a product containing two molecules of *N*α-acetyl-L-lysine-*N*-methylamide (expected at 487 m/z).

The structure of this product was determined by NMR spectroscopy. The ^{1}H-NMR spectrum of the product was free of resonances in the region expected for acryl protons, which eliminated the α-bromine substituted structure. Resonances due to aziridine protons were assigned to Hα (2.25 ppm) as well as Hβa and b (1.84 and 2.06 ppm) coinciding with the methyne resonance of the amino acid backbone. Confirmation of the aziridine structure was obtained from the ^{13}C-NMR spectrum of the isolated product. Resonances were not observed for acryl carbons, with all resonances in this region being assigned to aromatic carbons due to the benzophenone moiety of the product. Resonances due to the α and β-carbons of the aziridine ring were observed at 34.6 and 29.3 ppm, respectively. These shifts are consistent with those observed for the product of the reaction of 4-(α,β-dibromopropionamido)benzophenone [1] and the model dye mixture (**5** + **6**) with *N*α-acetyl-L-lysine-*N*-methylamide.

It was concluded that the reaction of the commercial Lanasol dye with *N*α-acetyl-L-lysine-*N*-methylamide resulted in a single product that contained an

aziridine ring. The mechanism of the reaction of **5**+**6** with *N*α-acetyl-L-lysine-*N*-methylamide would be analogous to that of the other primary amines considered in these investigations.

*Reaction of Lanasol Yellow 4G with N*α*-acetyl-L-histidine*

The purified product had a (−) ES–MS parent ion which displayed a complex parent ion (due to the isotopic splitting of bromine and chlorine atoms) at 425 m/z. A parent ion at this position was only expected for a product that contained an *N*α-acetyl-L-histidine molecule that had reacted at the β-carbon of the reactive group of the Lanasol dye via a Michael addition. This product is analogous to that obtained from the reaction the model dye mixture (**5**+**6**) with *N*α-acetyl-L-histidine. Parent ions were not observed for a product containing an *N*α-acetyl-L-histidine molecule that had substituted the α-bromine of the Lanasol dye (expected at 385 m/z) or for a product containing two molecules of *N*α-acetyl-L-histidine (expected at 483 m/z).

The structure of this product was confirmed by NMR spectroscopy. As anticipated, the ^{1}H-NMR spectrum of isolated product contained no acryl resonances, while the resonance at 6.85 ppm was assigned to a methyne imidazole proton. The region from 4.13 to 4.44 ppm integrated as two protons which were assigned to the CH resonance of the amino acid backbone of covalently bound histidine and to the resonance due to CH-Br. The structure of the product was verified using ^{13}C-NMR spectroscopy. Acryl carbons were not observed in the expected region of the ^{13}C-NMR spectrum of the product. The resonances in this region were assigned to the aromatic carbons of the dye carrier group and chromophore and to the imidazole carbons of the amino acid moiety of the product. The resonance at 49.2 ppm was characteristic of a CH-Br carbon and corresponded to that observed in the ^{13}C-NMR spectrum of the product isolated from the reaction of the model dye mixture with *N*α-acetyl-L-histidine.

It was concluded that the reaction of the commercial Lanasol dye with *N*α-acetyl-L-histidine resulted in a product that contained the amino acid bound via its secondary amine group to the β-carbon of the dye. No other products were observed.

Reaction of Lanasol Yellow 4G with N*-acetyl-L-cysteine*

The (−) ES–MS of the reaction mixture contained a parent ion at 368 m/z, which is expected for a product containing an *N*-acetyl-L-cysteine molecule attached to the β-carbon of the model dye while the α-carbon was reduced with the removal of the bromine. This structure is analogous to those obtained from the reaction of 4-(α,β-dibromopropionamido)benzophenone [1] and the **5**+**6** mixture with *N*-acetyl-L-cysteine. Parent ions were not observed for a product containing an *N*-acetyl-L-cysteine molecule that had

reacted at the β-carbon of the dye via a Michael addition without loss of the bromine from the α-carbon (expected at 408 m/z) or for a product containing two molecules of *N*-acetyl-L-cysteine (expected at 449 m/z). Of the reactants, a molecular ion for *N*-acetyl-L-cysteine was detected at 162 m/z, while the Lanasol dye (expected at 327 m/z) was not. These results are consistent with the TLC.

CONCLUSIONS

The findings from Part II of this study, which used conditions that simulate the commercial application of Lanasol dyes to wool, basically mirror those results obtained from the acetone/water investigation. The α,β-dibromopropionamido group was not found to be significantly dehydrobrominated when subjected to commercial dyeing conditions in the absence of model wool compounds. Both the α,β-dibromopropionamido and α-bromoacrylamido groups reacted with model wool compounds to form the same products. It was also found that the reactions of a mixture of model dyes (**5** + **6**) with *N*α-acetyl-L-lysine-*N*-methylamide, *N*α-acetyl-L-histidine and *N*-acetyl-L-cysteine resulted in only a single product for each reaction whose structure were analogous to that obtained for the corresponding reaction performed in acetone/water with 4-(α,β-dibromopropionamido)benzophenone. Thus, the presence of acetone in the solvent appears to have had no effect on the outcome of the reactions of these side chain groups. Under the simulated dyeing conditions used in this investigation, *N*-acetyl-L-serine and glycine were not reactive towards the model dye mixture (**5** + **6**). Glycine is probably not the ideal model for the *N*-terminal group of wool protein as it exists in water at pH 5 as a zwitterion. Products containing two amino acids covalently bound to one Lanasol reactive group were not observed for any of the model wool compounds tested. This was also the case when the reactions were performed in acetone/water at 65°C, which shows that the solvent composition and temperature did not have a significant effect on their reluctance to form.

A commercial Lanasol dye was reacted with *N*α-acetylated amino acids under conditions that simulate those used when these dyes are applied to wool. The products formed from the reactions of the dye with *N*α-acetyl-L-lysine-*N*-methylamide, *N*α-acetyl-L-histidine and *N*-acetyl-L-cysteine were analogous to those obtained using model dye compounds. Although this investigation was performed under the conditions that most closely simulate commercial dyeing, products containing two amino acids covalently bound to one molecule of Lanasol dye were not observed from any of the reactions tried. This provides strong evidence that significant amounts of

cross-links between Lanasol dyes and peptide chains within wool are unlikely to occur.

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