

AN INTERPRETATION OF THE MECHANISM OF UNHAIRING AS A NUCLEOPHILIC DISPLACEMENT*

WALLACE WINDUS AND JOHN S. SHOWELL†

*Eastern Regional Research Laboratory‡
Philadelphia, Pennsylvania 19118*

ABSTRACT

New concepts in organic chemistry have expanded the knowledge of the chemistry of divalent sulfur. It is now generally accepted that the cleavage of the sulfur-sulfur bond is brought about by a nucleophilic displacement rather than by an oxidation-reduction reaction. In unhairing, it is usually the sulfur-sulfur bond of the cystinyl residue in epidermis and hair that is attacked. This has been overlooked in the scientific literature.

In this paper, nucleophilic displacement is proposed as the key step in the mechanism of unhairing in which the sulfur-sulfur bond is cleaved. Supporting evidence from the literature is presented and unhairing with amines interpreted as a displacement reaction, giving a sense of unity to the mode of action of various unhairing agents.



INTRODUCTION

Organic chemistry has changed a great deal in the last 35 years, as those of us in the older generation are all too well aware. The products of a reaction may have remained the same, but the interpretation of the pathway by which they are formed has often changed beyond recognition. Organic chemistry has been clarified so that new relations are apparent. This is not confined to compounds of carbon, hydrogen and oxygen but includes organic sulfur compounds.

In spite of the mention in recent scientific literature of many practical application of the cleavage of the sulfur-sulfur bond, its significance in unhairing has been overlooked. One would get the impression that the leather industry does not exist! In view of the rapid advances in the theoretical understanding of the chemistry of divalent sulfur, the mechanism of unhairing deserves re-examination.

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†Now with the National Science Foundation, 1800 G Street, N. W., Washington, D. C.

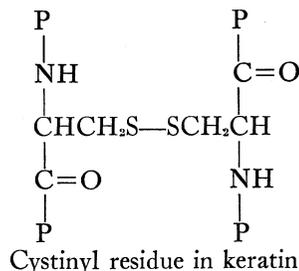
‡Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

The disulfide with which we are concerned is the cystinyl residue in epidermis and hair. The free amino acid has the following formula:



Cystine

The segments of the peptide chains in keratin that contain the cystinyl residue can be abbreviated to:



It is now accepted that in keratin the two halves of the cystinyl residue are linked in different polypeptide chains, in most instances. This increases the stability of a certain conformation of the protein, with the exception that this structure increases its vulnerability to chemicals that cleave the sulfur-sulfur bond. The amino and carboxyl groups in cystine withdraw electrons from sulfur. This polarizes the sulfur-sulfur bond and, in effect, weakens it. Electron withdrawal is even greater in the protein than in the free amino acid (1). The formula will be still further abbreviated throughout the rest of the paper to:



Atkin and Thompson (2) were apparently the first to suggest that sulfides reduce the cystinyl residue of epidermis and hair to a cysteinyl residue. The free amino acid has the formula:



Cysteine

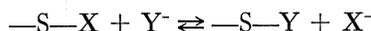
This hypothesis led to the discovery of the unhairing action of stannous chloride by Merrill (3), of sodium cyanide by Marriott (4), and of mercaptans or thiols by Turley and Windus (5, 6). While this explanation was obviously useful and the end products remain the same, the reduction theory of unhairing is now somewhat dated.

There has been some question in the past as to whether it is correct to assume that the epidermis and hair are similar in composition, even though both are considered to be keratin. It is now established that both contain a substantial amount of cystine. Epidermis differs from hair and wool in having a lower content of cystinyl residues and a higher content of cysteinyl residues. The lower stability of epidermal keratin to physical and chemical agents is attributed to fewer sulfur-sulfur cross-links (7, 8). Differences in physical structure between epidermis and hair must also be taken into account. Some other cross-links, such

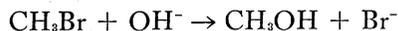
as hydrogen bonds and salt links, do exist and are broken by alkaline solutions, but these are weak and are a minor factor in stabilizing the keratin structure. It is, therefore, legitimate to concentrate our attention on the cleavage of the sulfur-sulfur bond. This is brought about by all of the standard commercial methods of unhairing.

NUCLEOPHILIC CLEAVAGE OF THE SULFUR-SULFUR BOND

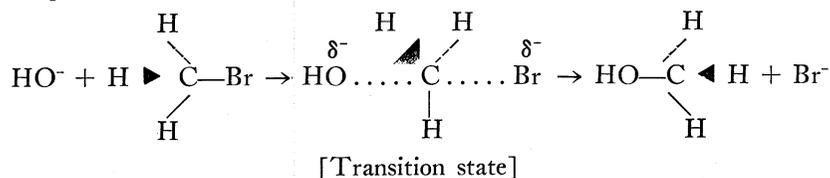
Olav Foss (9) was the first to recognize the common basis for the cleavage of a sulfur-sulfur bond in different inorganic and organic compounds by a wide variety of chemicals. He characterized the reaction as a nucleophilic displacement of a thioanion from sulfur by a nucleophile which has a greater affinity for sulfur than the displaced thioanion. As early as 1876, Schiller and Otto (10) showed that the sulfur-sulfur bond in diphenyldisulfide was cleaved by potassium hydroxide. This is simply a special case of nucleophilic displacement from a divalent sulfur atom. The unusual feature is that two sulfur atoms are linked to each other and are available for attack by a nucleophile. A general statement of the reaction is:



To review the terminology briefly, the concept of nucleophilic and electrophilic reactions was developed largely by a monumental work of Ingold and his associates (11). A typical nucleophilic displacement is the hydrolysis of methyl bromide to methyl alcohol.



A nucleophile is a molecular species that donates its electrons to, or shares them with, an atomic nucleus. The word "nucleophile" means "nucleus-loving," since the negatively charged nucleophile is seeking the positive charge of a nucleus. The next equation is a modern representation which shows the transition state.

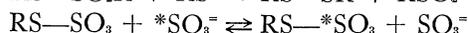
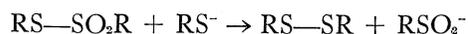
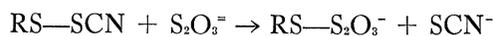


Broken lines indicate bonds to atoms lying behind the plane of the paper and solid triangles to those lying in front of it. The dotted lines indicate partial bonds and δ^- partial valences.

The first stage in the transition state is the slower part of this reaction and therefore determines the rate. The molecularity of a reaction is defined as the number of chemical species that form new bonds or suffer the breakage of old bonds during the rate determining step. In this reaction two molecular species are involved in the first step, so the reaction is bimolecular. This is abbreviated to $\text{S}_{\text{N}}2$, namely substitution, nucleophilic, bimolecular.

The term base is used in the broad sense proposed by G. N. Lewis (12); namely, any group that can donate a pair of electrons. Basic species are often called nucleophilic reagents or nucleophiles. However, basicity and nucleophilicity may or may not parallel each other.

Nucleophilic displacements are now well known. The following are examples (1):



The last equation is particularly interesting because a sulfite anion is displacing a sulfite group. The reaction can only be followed by using isotopic sulfur which is radioactive, as indicated by the asterisk.

UNHAIRING

The authors are proposing that this nucleophilic displacement is the key step in the mechanism of unhairing. It is no longer correct to say that unhairing is a reduction reaction. Rather, it is a displacement reaction. This may seem like an argument in semantics, but it has some broad implication. Quite aside from increasing our understanding and providing a wider scope for our thinking, it permits the correlation of diverse reactions that have appeared to be unrelated. They can now be explained by one mechanism. It also supplies a mechanism for the action of one group of unhairing agents for which no plausible mechanism has been proposed. Finally, it may lead to the recognition of new unhairing agents.

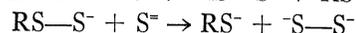
Sulfides

Under sulfides we can include all water-soluble, inorganic sulfides and any that can be made to react to form a soluble sulfide. This will then include arsenic sulfide, sodium sulfide, calcium sulfide and the sulphydrates of sodium and calcium, since the latter are converted to sulfides by calcium hydroxide. The chemistry of sulfide unhairing owes much to the work of Procter (13), Stiasny (14), Merrill (7), and Marriott (4).

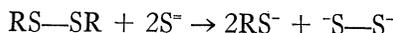
In 1886, Otto and Rössing (15) showed that the sulfur-sulfur bond of diphenyldisulfide, di-p-tolyldisulfide and dibenzylidisulfide was cleaved by potassium sulfide in warm alcoholic solution. The first compound is adequate to illustrate the reaction.



The reaction can now be represented as a two-step nucleophilic displacement as follows:



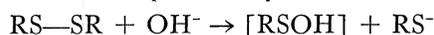
which in summation is:



While the sulfide anion is the unhairing agent, the alkalinity is important. No unhairing takes place below a pH of approximately 12.0. The concentration of a nucleophile is increased by an increase in alkalinity. Unhairing liquors with their high pH are ideal for generating a relatively high concentration of the nucleophile from a limited amount of the "sharpening agent." Many unhairing procedures are similar in that they involve a dilute aqueous solution at room temperature. This increases the validity of a direct comparison of different nucleophiles. The mild conditions enforced by the properties of collagen are probably responsible for the necessity of a relatively high pH for unhairing. Many synthetic disulfides can be cleaved below a pH of 12 in the laboratory by using a concentrated solution of a nucleophile at an elevated temperature. One must also remember that proteins are polyelectrolytes, so that the conditions within the protein may not be identical with those in the external solution. The advantage of lime is, of course, that it provides a uniform and dilute solution with a high pH, which is desirable for the "liming" of the collagen as well as for unhairing.

The presence of calcium cation or a mixture of calcium and sodium cations is probably important for another reason. Alkyl thiols are insoluble in water, but their calcium and sodium mercaptides are soluble. In most unhairing reactions which depend upon the cleavage of the sulfur-sulfur bond at least one cysteinyl residue is formed. The cysteinyl residue is a thiol whose solubility is increased by conversion to the calcium and sodium mercaptides. The solubility of the epidermis is also increased by a higher content of cysteine in proportion to the total sulfur that exists in hair. Cysteine is converted directly to the mercaptide without the necessity of cleaving a sulfur-sulfur bond. This is particularly true in the growing lower layer of epidermis referred to as "young-keratin" or "prekeratin."

Hair is often dissolved by the use of a relatively high amount of sodium sulfide in the absence of lime. This is not a contradiction since a sodium sulfide solution is strongly alkaline and has a high pH, in contrast to a solution of sodium sulfhydrate which is not an unhairing agent in the absence of lime because the pH of an aqueous solution is under 12. While it is not done commercially, one can dissolve hair in very strong sodium hydroxide solution. This is probably an example of the reaction much emphasized by the Schöberl school (16), namely,

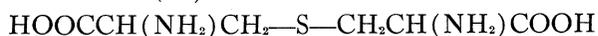


With the possible exception that it may occur as a minor side reaction, it does not take place in commercial unhairing because the hydroxyl anion is a weak S-nucleophile and the concentration of hydroxyl anion is relatively low. This would lead to a different reaction in the absence of a strong nucleophile. The unhairing action of lime is discussed later. In a borderline case, such as sodium sulfite, it is hard to decide whether the slight acceleration of unhairing is due to the sulfite anion or to the increase in alkalinity resulting from the reaction of the sodium salt with calcium hydroxide to yield sodium hydroxide. In any event, sodium sulfite is not a practical unhairing agent.

Formation of Lanthionine

There is one undesirable reaction that can happen in a tannery through carelessness. It has been known by tanners probably as long as sodium sulfide has been used with lime for unhairing. If calcium hydroxide is allowed to act on hides or skins for even a few hours before the sulfide is added, the unhairing is retarded. If the skins are immersed in a suspension of lime alone overnight, no amount of sulfide added the next day will dissolve the hair. This has become known as "immunization." Alan Rogers was the first to state clearly that, for maximum unhairing action, all of the sulfide should be added at the beginning (17).

The explanation of immunization was provided by Horn, Jones and Ringel (18). They found that the cystinyl residue of wool is converted to a new amino acid residue when wool is boiled in a two percent sodium carbonate solution for one hour. The new amino acid was isolated and named lanthionine. They and du Vigneaud and Brown (19) established the structure as:



Lanthionine

Cuthbertson and Phillips (20) have shown that the cystinyl residues of wool are converted to lanthionyl residues very rapidly by a solution of barium hydroxide at room temperature. In effect, one atom of sulfur has been removed from the cystinyl residue. The mechanism is still uncertain (21). This is a very stable thioether link and is not cleaved by S-nucleophiles.

It is remarkable that "despite the ease and efficiency with which disulfide linkages in protein can be transformed into thioether linkages there is not one authenticated case of a thioether resulting from the action of alkali alone on a disulfide other than one which is an integral part of a protein (21)."

The use by Somerville and Hetzel (22) of a divalent cation, such as that supplied by calcium chloride added ahead of the dimethylamine-sodium hydroxide unhairing system, is an interesting application of "immunization" for the protection of the hair. The formation of lanthionyl residues by the action of calcium hydroxide formed after the hides or skins are subjected to the caustic unhairing liquor is rapid. This is indicated by the fact that the protective action is more effective if the dimethylamine sulfate and sodium hydroxide are added directly to the liquor containing the hides and calcium chloride, rather than transferring the hides to a separate liquor (personal communication from I. C. Somerville). The speed of the transformation by the calcium hydroxide may be attributed to the equivalent of an *in situ* reaction. It is apparent that there is a careful balance between immunization and unhairing. The protective action can be overwhelmed by the addition of more sodium hydroxide unless a further adjustment is made.

It seems likely that some lanthionyl residues are formed under most unhairing conditions, with the possible exception of a hair pulping system in which the

cleavage of the sulfur-sulfur bond is rapid and probably complete due to a large excess of the sulfide anion.

An unhairing system is complex. The main reaction is often partially reversible; the products of the initial reaction take part in secondary reactions and side reactions occur. Tanners have always been adept at suppressing unwanted reactions in favor of the desired reaction. Compromises are sometimes necessary in the interest of correct "liming" of the collagen which takes place simultaneously.

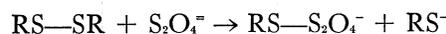
It is well known that hides and skins can be unhaird with lime alone, but this is slow and is caused mainly by the hydrolysis of the peptide or amide groups and the formation of the calcium mercaptide from cysteinyl residues present in epidermis, but not in hair, as mentioned earlier.

As a corollary, it would probably be preferable to use a small amount of sodium sulfide to accelerate soaking, rather than a small amount of sodium hydroxide although the action of sodium hydroxide is less rapid and specific than that of calcium hydroxide. However, the amount is usually small and is partially neutralized by the collagen so that only a few thioether links can be formed, and there is no serious interference with unhairing. One cannot, however, use calcium hydroxide to accelerate soaking.

A caustic soda or lime pretreatment does not retard enzyme unhairing. This is additional evidence that enzyme unhairing does not depend upon the cleavage of the sulfur-sulfur bond.

Dithionites or Hydrosulfites

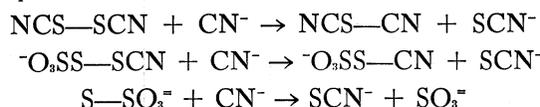
Sodium dithionite, better known in commerce as sodium hydrosulfite, is in use as an unhairing agent (23). Its activity is less than that of sulfides since it will not dissolve hair. However, it is more reactive than sodium sulfite which has little or no unhairing action and is not used commercially. This means that the dithionite anion is a sufficiently powerful S-nucleophile to displace a thioanion from a disulfide. The initial reaction is a straightforward nucleophilic displacement.



It is curious that no reference could be found in the literature involving the action of this anion on any disulfide even though the less S-nucleophilic sulfite anion has been studied extensively on many synthetic disulfides.

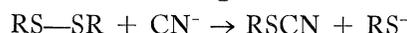
Cyanides

The cyanide anion of sodium and potassium cyanide is known to cleave the sulfur-sulfur bond in a number of organic compounds by nucleophilic displacement. Some examples are as follows:



It is significant from the standpoint of leather chemistry that simple dialkyldisulfides do not react with cyanide anion (24), but often do so when carboxyl and/or amino groups are present due to electron withdrawal from the sulfur atoms by these groups. For example, cystine with cyanide yields cysteine and cyanocysteine initially (25, 26). The cyanocysteine cyclizes to a ring structure under suitable conditions (27, 28). Gawron (29) has reviewed the reaction of cyanide with cystine and cystine peptides.

Sodium cyanide is in use for unhairing and the initial reaction is as follows:



The cyanide anion is a sufficiently powerful S-nucleophile to dissolve hair.

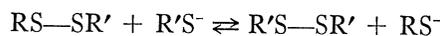
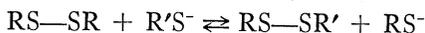
It was incorrect to refer to cyanides as reducing agents, since they are not. The fact that they accelerate unhairing should have provided a clue that something was amiss with the "reduction" theory of unhairing.

Thiols

Turley and Windus (5, 6) discovered the unhairing action of thiols in 1932. The hair can be saved or destroyed, depending upon the amount used. Many thiols are active, although the simple aliphatic thiols such as butylthiol and thioglycolic acid are most active. At about the same time, Goddard and Michaelis (30) showed that chicken feather keratin, hair and wool, are dissolved by an excess of thioglycolic acid in alkaline solution. In spite of the very disagreeable smell of thiols, the calcium salt of thioglycolic acid has displaced calcium sulfide as an underarm depilatory and is the active ingredient in the cold-waving of hair (31).

In accordance with the work of Lecher (32), the reaction was described as an exchange reaction, and it was indicated that it was not a typical oxidation-reduction reaction (6). It was later shown that benzylthiol was essentially quantitatively converted to dibenzyldisulfide during unhairing under nitrogen (33). However, the paper was entitled "A Proof of the Reduction Theory of Unhairing." It is now generally agreed that it is not oxidation-reduction in the sense of a radical cleavage of the sulfur-sulfur bond but is rather a two step anionic displacement (34, 35).

The reaction is especially interesting because one thiol is displacing another thiol. The displacing thiol anion is more basic or S-nucleophilic than the thiol being displaced. The mechanism is indicated by:



which, in summation, is



Mixed disulfides have been isolated, confirming the two-step mechanism (36, 37). The first reaction may be adequate to represent unhairing; but, since the second reaction usually rapidly follows the first, the question is academic. Again, the

formation of the calcium mercaptide increases the solubility of the fragments of epidermis without apparent change of the hair in a hair-saving process.

The above reactions have been written as reversible, and the point of equilibrium is determined by the respective S-nucleophilicity of the $R'S^-$ and RS^- . There is a close correspondence between the ease of oxidizing $R'S$ to $R'S-SR'$ and the S-nucleophilicity of the thiol. The lower the electrode potential of the system $2R'S/R'S-SR'$, the greater the S-nucleophilic strength of $R'S^-$.

Vago (38), was apparently the first to emphasize the importance of the reduction potential in unhairing. He postulated that this value should be -0.3 to -0.4 volts in order for a compound to be an unhairing agent. The value for $1M$ sodium sulfide solution is -0.56 volts. He recognized that sodium selenide and telluride with reduction potentials of, respectively, -0.75 and -0.91 volts in a $1N$ solution should be unhairing agents. He dissolved hair with sodium selenide.

Turley and Windus (6) have shown that cysteine and reduced glutathione accelerate unhairing. The cysteine anion, $HOOC-CH(NH_2)CH_2S^-$, is particularly interesting because it accelerates unhairing by displacing itself, so to speak. One must visualize that the sulfur-sulfur cross-link between polypeptide chains is broken. One chain then contains a cysteinyl residue which terminates as a thiol group. The calcium cation must increase the solubility of the thiol and aid unhairing by formation of the calcium mercaptide. This cleavage is not surprising when it is recalled that a sulfite anion in which the sulfur is an isotope has been shown to displace the sulfite group in a thiosulfate.

The fact that aromatic thiols are not unhairing agents is also clarified by this displacement mechanism. Relatively small changes in S-nucleophilicity can cause the difference between hair pulping and no unhairing. Dissociation constants, which also correlate to a degree with S-nucleophilicity, can be cited to show a difference. *n*-Butylthiol has a pK_a value of 11.5 and *n*-hexylthiol a pK_a value of 13.5. However, thiophenol has a value of approximately 8 (39).

One example will illustrate the usefulness of modern electronic theory. In an attempt to find an aromatic thiol that would be an unhairing agent, a number of substituted thiophenols were tested. One compound in particular, the para-nitrothiophenol, was a poor choice. The nitro group is a strong electron-withdrawing group. This decreases the S-nucleophilicity of the thiol group, which indicates that there was no point in testing this compound.

Amines

In 1927, McLaughlin, Highberger and Moore (40, 41) announced their discovery that primary aliphatic amines with lime accelerate unhairing. In unpublished work, Turley determined that dimethylamine, a secondary amine, is roughly twice as active as monomethylamine. This was confirmed by Moore, Highberger and O'Flaherty (42). Trimethylamine, a tertiary amine, does not accelerate

The unshared pair of electrons on the nitrogen atom has been indicated, since these electrons are responsible for the amine acting as a nucleophile and cleaving the sulfur-sulfur bond. However, in water solution an amine is protonated as follows:



A strong base such as calcium hydroxide or sodium hydroxide, must be present to generate the free amine.



This is analogous to ammonia.



Nucleophilic displacement provides the first reasonable and unifying mechanism for amine unhairing. In the past, this reaction has appeared to be unrelated to sulfide, thiol and cyanide unhairing.

In addition, by its use, an explanation can be offered for the difference in reactivity of ammonia, mono-, di- and trimethylamine in unhairing in a calcium hydroxide solution. Within an homologous series, it is legitimate to relate S-nucleophilicity to basicity. The basicity of amines is dependent upon the availability of the unshared pair of electrons on nitrogen. Basicity increases from ammonia to dimethylamine. The pK_b values are: ammonia 4.75, monomethylamine 3.36, and dimethylamine 3.23. The lower value indicates greater basicity. The pK_b value of trimethylamine is 4.20 (47).

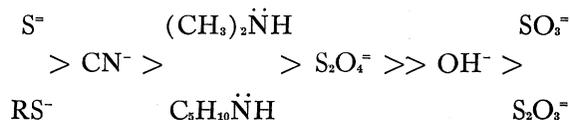
This data indicates that the S-nucleophilicity of dimethylamine is greatest and that of ammonia least. It is apparent that a small difference between the basicities of mono- and dimethylamine causes a substantial difference in their unhairing activity, and a further reduction of basicity causes a complete loss of unhairing activity in the case of trimethylamine. Merrill (47a) has pointed out that a moderately strong solution of ammonia, in the absence of lime, produces a relatively rapid hair-slip on fresh calfskins. In our tests on salt-cured hides the action was slow.

Of several additional amines that accelerate unhairing, piperidine is most active and is comparable to dimethylamine. It is not only strongly basic, but the hydrocarbon part of the molecule is said to be "tied-back" from the nitrogen atom. The nitrogen atom can therefore more easily attack the sulfur atom to form the transition state, with a minimum of steric hindrance from the rest of the piperidine molecule. Monoethyl and diethylamines are weak unhairing agents even though their basicities are higher than those of the corresponding methylamines. The pK_b values are 3.33 and 3.07, respectively. This is probably due to the size of the alkyl groups, which can rotate freely about the nitrogen atom and cause steric hindrance to attack on the sulfur atom. This explanation is plausible

for diethylamine since there is also much steric hindrance within the protein structure. However, it may be somewhat less satisfactory for monoethylamine.

ORDER OF UNHAIRING ACTIVITY

From practical experience, it is a simple matter to list unhairing agents in decreasing order of their activity as follows:



This indicates their S-nucleophilicity with respect to the cleavage of the sulfur-sulfur bond in the cystinyl residue in epidermis and hair. The first three on the left are such strong S-nucleophiles that they are capable of dissolving hair when used in moderate amounts with lime. The position of cyanide anion may be somewhat arbitrary and is based upon evidence in the theoretical literature that it is a weaker S-nucleophile than the sulfide and thiol anions. Dimethylamine will dissolve hair when used in moderate amounts with sodium hydroxide, but not with calcium hydroxide (22). The dithionite anion will not dissolve hair, at least when used in any practical amount. The hydroxyl anion is always described as a weak nucleophile. In any event, when used alone in the absence of a strong nucleophile, it causes the formation of the lanthionyl residue, as previously explained. The sulfite and thiosulfate anions, if they possess any unhairing activity at all, are such weak S-nucleophiles that they are not practical unhairing agents.

In the main, this order parallels various rankings of S-nucleophilicity given in the literature (48, 49, 50). However, these references show a few anomalies. Sulfide anion is omitted, although it is well known to be a powerful S-nucleophile. Sulfhydrate anion is included in one list, whereas it is converted to the sulfide anion in alkaline solution. Aliphatic amines are omitted in all lists although they are mentioned in one text. Dithionite anion appears to have been overlooked by all academic research workers. Phenylthiol anion from thiophenol is placed ahead of cyanide anion in two lists although it had no unhairing activity. Some other anions are active S-nucleophiles under special laboratory conditions, but are not unhairing agents because they are insoluble or unstable in water.

SUMMARY

In summary, nucleophilic displacement has been proposed as the key step in the mechanism of unhairing in which the sulfur-sulfur bond is cleaved. Supporting evidence from the literature has been presented. Unhairing with amines has been interpreted as a displacement reaction. A sense of unity in the mode of action of various unhairing agents has been attained. It is apparent that there are a number of areas that would profit from additional research.

ACKNOWLEDGMENTS

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DISCUSSION

CHAIRMAN: The discussion of this paper will be led by Dr. Prentiss.

DR. PRENTISS: A significant contribution is always made when the net result is to increase our understanding. We believe that this correlation of the various unhairing mechanisms certainly does increase our understanding and for this we are appreciative to Dr. Windus.

One thing that goes through my mind when I look at the various mechanisms of unhairing is that in practice there is always a question as to whether hair will

dissolve or not. I ask this question in regard to hair immunization, and I would ask Dr. Windus if he would describe how these nucleophilic displacement reactions fit into the immunization concept of unhairing.

DR. WINDUS: Immunization is very interesting. There simply wasn't time to cover it in twenty minutes. To cite an example, if hides or skins are put in lime alone in water with no unhairing accelerator for even one or two hours before the sulfide is added, unhairing is definitely retarded. Every practical tanner knows this. If they should, by chance, be left in the lime suspension overnight, and the sulfide added the next day, no amount of sulfide will dissolve the hair. This has become known as immunization.

The explanation was forthcoming only about twenty years ago by Horn, Jones and Ringel, working, by the way, in the Department of Agriculture, in Beltsville. They reacted alkali with wool, hydrolyzed the protein and found that they had a new amino acid, which they named lanthionine.

Can you project the A slide? You see the only thing missing here in comparison with cystine is one atom of sulfur. Of course, it is not that simple. One doesn't just pull out an atom from the middle of a molecule. The mechanism is still uncertain. There are at least three theories, none proven. This is a thioether link which is very stable and is not cleaved by S-nucleophiles. To obtain maximum unhairing action, as Allan Rogers stated many years ago, all of the sulfide should be added at the beginning, either with or slightly in advance of the lime. This at least reduces the formation of lanthionine to a minimum.

DR. PRENTISS: Are there any questions from the audience? Dr. Windus, you listed on one of your slides the activities of the various nucleophiles in terms of their strength of unhairing. Each of these is used in one way or another in practice for unhairing. Would you give us some explanation as to why one might be used under one set of circumstances over another?

DR. WINDUS: Well, that's a broad question. Sometimes I think it is a matter of personal choice, but that is an oversimplification. First, the paper was confined to unhairing and I omitted liming deliberately. As far as the collagen is concerned I've long thought that there is some mild, controlled hydrolysis of peptide links in the collagen. As you saw in the example of methyl bromide to methyl alcohol, hydrolysis is a nucleophilic displacement by the hydroxyl anion.

Now when it comes to a choice, you decide that partly on the basis of the leather you are making and on the raw stock being processed. As an extreme example, if you want to dissolve the hair, you would use, historically, a high amount of sodium sulfide. If you want the maximum beauty from a smooth grain with minimum distortion of the hair cells, as in aniline finished calf, you use a mellow liming system for the other extreme. This is why arsenic sulfide and lime lasted

so long in the calfskin branch of the industry. In between it does become somewhat a matter of choice.

DR. PRENTISS: Thank you, Dr. Windus. Any questions? One of the things that you mentioned early in the paper, Dr. Windus, was the influence of pH. Are there conditions other than this which generate nucleophiles which would also cause unhairing?

DR. WINDUS: Yes, there was one disturbing thing as I studied the literature. In a number of reactions that were given for synthetic disulfides the sulfur-sulfur bond was cleaved below a pH of 12, and yet we know that unhairing does not take place below this pH. However, in unhairing, our conditions are extremely mild; a dilute aqueous solution at room temperature. That is because we have no choice due to the properties of collagen.

Laboratory reaction conditions are quite often drastic, such as boiling under reflux. The nucleophile may be present in enormous concentration, a hundred times the amount of the nucleophile that we use in unhairing, for example.

Apparently, we have to have a high pH to generate the maximum concentration of the S-nucleophile from the unhairing agent. The nucleophile is not made more active by a high pH. Once it is generated it is active, but it has to be present in maximum concentration from the small amount of the unhairing agent and that is brought about by a pH above twelve.

DR. PRENTISS: Dr. Windus, there is one common residue in the keratin of the epidermal tissue and the keratin of the hair itself and that is cystine. These nucleophilic reactions all attack the cystine. How do we distinguish between the types of reactions which would occur in the epidermal tissue versus those which would directly attack the hair? In other words, under some conditions you get hair pulping or hair destroying; with the same chemicals you can get strictly loosening of the hair or its removal intact.

DR. WINDUS: I covered that rather hurriedly, Dr. Prentiss, but there is good evidence that there is more cysteine (e-i-n-e) in epidermis and probably little or none in the hair because the hair is a somewhat mature structure. Of course, that may not apply to the hair root and the growing bulb of the hair.

The literature on the growth of hair and its structure indicates that the epidermis and the hair root are more susceptible to attack because of the existence of the pre-keratin, in which the —SH group in cysteine has not yet been oxidized to the —S—S— group of cystine which must be cleaved. The RSH is converted directly to calcium or sodium mercaptide which increases its solubility.

The concentration of the cysteine is greatest in the lower layer of the epidermis. If you solubilize the lower layer you simply lift off the epidermis.

Accordingly, it is usually a matter of the amount of nucleophilic agent you use whether you get hair saving or hair destruction.

DR. PRENTISS: It was interesting to note that in the first slide you talked about hydroxide attack on the disulfide bond and then proceeded to discuss the various nucleophilic agents. What is the distinction between the alkali, say as an alkaline hydrolysis, and the other types of reactions?

DR. WINDUS: If, for the sake of the argument, we ignore the formation of lanthionine and assume there can be hydrolysis of the keratin by a weak nucleophile, namely the hydroxy anion, this is what would take place with lime or sodium hydroxide alone. The reaction — can you show slide two please — the reaction is the first one on this slide.

For a number of years, Schöberl in Germany and others have postulated this reaction as the initial reaction in the cleavage of a disulfide. I myself think that the emphasis on this reaction actually delayed recognition of nucleophilic displacement in the cleavage of the sulfur-sulfur bond. To me, it isn't reasonable to assume that this hydrolysis is the first reaction in unhairing because it is a slow reaction and would govern the rate of unhairing. The reason you add unhairing agents is to accelerate unhairing. Accordingly, you suppress or eliminate this reaction by adding sulfide, amines, and so on.

Finally, if you have lime alone you get into the involved question of the formation of lanthionine which probably takes preference over formation of the sulfenic acid.

DR. PRENTISS: Are there any further questions? If not, then we thank you again, Dr. Windus, for this very explicit and interesting paper.
