LXXVII. ON A SERIES OF METALLO-CYSTEIN DERIVATIVES. I.

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WHEN a stream of air is blown through an alkaline solution of cystein, the oxidation is generally accompanied by the production of a violet coloration; an observation that will be familiar to workers who have had occasion to prepare cystine in this manner. Although the phenomenon has been long known its cause has remained obscure, and the present investigation was undertaken primarily with the object of ascertaining its nature.

The fact that cystein gives with ferric chloride and ammonia an intense coloration, which becomes darker on shaking, was first noticed by Andreasch [1884]. Arnold [1911], describing a number of tests by which cystein may be recognised, distinguishes between the colour produced with ferric chloride and that with ammonia. "If a solution of cystein is made weakly alkaline a violet colour is produced which is more permanent than that given by sodium nitroprusside...." There can be little doubt however that the coloration is in reality due not so much to the action of the alkali upon the cystein, as would appear from Arnold's account, as to the presence of traces of a metallic (generally ferric) salt, which produce the typical coloration in alkaline solution. Taking care to ensure the complete absence of a metallic ion I have been unable to observe the production of the slightest colour.

An important feature of the reaction is its impermanent character. Arnold observes that the colour fades after a while, but can be renewed by shaking. The nature of this curious alternation of fading and restoration of colour receives no explanation from Arnold.

The Reversible System: Cystein—Iron—Ammonia.

When cystine is isolated by the use of mercuric sulphate reagent, the Hg-cystine compound which is precipitated, yields, on decomposition with H_2S , a solution of the reduced cystein. This can be re-oxidised, after removal of H_2S , by making alkaline with ammonia and blowing a stream of air through the solution. Under these circumstances there is generally enough iron present

as impurity to produce a noticeable violet coloration in the solution as soon as the active oxidation is started. Often a state of delicate equilibrium is set up in which the colour disappears upon the air current being stopped, and immediately reappears upon its continuation. The necessary conditions may be reproduced artificially by adding to a test tube containing (say) 4 cc. of a 5 % cystein solution a single drop of weak FeCl_3 solution. Upon making alkaline with weak NH_4OH , a violet colour appears, only to fade almost immediately. The colour reappears if the tube be shaken, or if air (or, better, oxygen) be otherwise brought into intimate contact with the solution. Bubbling hydrogen, carbon dioxide or nitrogen through the liquid has no effect.

Variation in the concentrations has a visible effect on the course of the phenomena. The depth of colour produced is directly proportional to the quantities of both cystein and ferric chloride present together. Again, the greater the concentration of ferric chloride relative to that of cystein, the longer does the violet colour survive the cessation of the air current, or, to look at it in another way, increasing the proportion of cystein in the mixture, brings about a more rapid fading when the air current is stopped. The solution remains violet (or alternatively capable of yielding a violet colour on aeration) so long as unoxidised cystein is present; the presence of the latter can be demonstrated by the extremely delicate nitroprusside test¹. When oxidation is complete the solution consists of cystine together with a minute quantity of $\text{Fe}(\text{OH})_3$, derived from the added iron salt. The effect of increasing the alkalinity is to yield a solution which less readily decolorises in the absence of the air current, but in which complete and permanent loss of colour (or ability to produce it on addition of the iron salt) occurs more rapidly, owing apparently to the greater rapidity of the transformation cystein $\longrightarrow$ cystine in the more alkaline solution.

Numerous ferric salts, also suspended ferric hydroxide, have been investigated as substitutes for FeCl_3 . All were equally efficient. Potassium ferri-cyanide was without action.

Explanation of the Phenomena.

Several explanations might be adopted to account for the observations described above. At first sight it would seem likely that the colour is due to the formation of an unstable compound in a state of oxidation intermediate between cystein and cystine; its presence being made manifest as a result of its power to yield a coloration with ferric salts.

Secondly, it may be assumed that direct oxidation of cystein to a body of the cystine peroxide type occurs, and that this hypothetical substance is reactive to FeCl_3 . The consecutive reaction is between cystein and the peroxide to yield the end product, cystine. There are theoretical grounds for postulating the possible existence of such a peroxide.

¹ It is possible to fix approximately the completion of oxidation by the disappearance of the violet coloration.

In view, however, of similar reactions between cystein and salts of certain other metals, notably copper, the following explanation would appear to fit the facts more closely than either of these.

Cystein is an acid, and it may be supposed that in the presence of Fe^{+++} ions, and in alkaline solution, it forms a violet-coloured ferric derivative. The main characteristic of cystein is its reducing power:

If excess is present and the supply of air be limited the cystein is oxidised to cystine at the expense of the ferric ion, which is thereby reduced to the ferrous state. The latter yields no coloured derivative with cystein, and the solution therefore fades. If now air be blown through the mixture, the Fe^{++} is rapidly re-oxidised to Fe^{+++} , Fe^{++} as is well known being unstable in alkaline solution when available oxygen is present. The colour is therefore regenerated provided some unoxidised cystein remains to form the anion of the coloured body, and it will be retained until the process of oxidation is complete. In the presence of abundance of free oxygen the cystein will be oxidising continuously, and the iron will be maintained in the ferric condition.

This explanation accounts for the following experimental observations:

- (1) The intensity of colour on making alkaline with ammonia is proportional to the amounts of cystein and Fe salt present together (*i.e.* the amount of cystein iron derivative produced).
- (2) Excess of cystein causes a more transient coloration (since the Fe^{+++} is more rapidly reduced in the presence of excess of the reducing agent).
- (3)₁ Excess of iron causes a more stable coloration.
- (4) Excess of alkali causes the coloration to be less *transient* (since Fe^{++} is increasingly unstable in alkaline solution).

Apparently the reduction potential of cystein does not develop with increasing alkalinity so rapidly as that of $\text{Fe}(\text{OH})_2 \longrightarrow \text{Fe}(\text{OH})_3$.

(5) With increasing alkalinity the *total duration* of the colour phenomena is less, since the cystein disappears more rapidly. (Cystine becomes more readily autoxidisable with increase of p_{H} .)

(6) It is found that the addition of *free cystein* to the coloured solution aids the (temporary) decolorisation. (Explanation as in (2).)

(7) It is a well known experimental fact that iron acts as a catalyst in accelerating the oxidation of cystein to cystine. This is readily explained on the basis of the above theory: the iron acts as a carrier; in the ferrous state it is a more efficient oxygen acceptor than the cystein, and oxidised it functions as an oxygen donator.

(8) The most convincing support to these views is afforded by the behaviour of copper (*vide infra*). It is found moreover that reversible colorations analogous with that given by iron could be obtained only by those metals which exist in more than one state of oxidation, and in which change of valency from the one form to the other can occur with tolerable ease.

With regard to the catalytic action of iron (see (7) above) mention should be made of the work of Mathews and Walker [1909] on the velocity of spontaneous oxidation of cystein in the presence of salt solutions. This paper was published prior to that of Arnold and I was unaware of the explanation therein advanced of the mechanism of the catalysis when the work described in the present paper was carried out.

"An explanation is given" by Mathews and Walker "of the action of different metals based on their solution pressure." These authors account for the catalytic action by assuming the momentary formation of an intermediate metallic compound

It would seem that this view fails to account for the experimental observation that the coloured derivative is only instantly decomposed when excess of free cystein is present. The pure cupric cystein derivative can be isolated and shows no tendency for internal spontaneous scission such as is suggested.

The action of cystein upon the cupric and other coloured metallic derivatives, can leave little doubt that the mechanism of the reaction consists in the reduction of the oxidised metallic atom of the derivative by the action of excess of cystein.

The formation of various metallic derivatives of cystein.

Fe⁺⁺⁺. The behaviour of iron is explained above. The deep coloration obtained when (excess of) ferric chloride is added to cystein was first described by Andreasch, and then by Arnold. The latter author distinguished between the colour given by FeCl₃ and by "ammonia." With sufficiency of iron the colour remains until oxidation of cystein to cystine is complete, and cannot then be regenerated by aeration.

Fe⁺⁺. Under ordinary conditions Fe(OH)₂ cannot be precipitated sufficiently free from Fe(OH)₃ to prevent the production of the violet ferric compound. Investigations have been started under anaerobic conditions.

Double salts (ferric alum, etc.) in which iron functions as Fe⁺⁺⁺ act like FeCl₃ or Fe(OH)₃. When iron functions in the complex anion as in K₄FeCy₆ or K₃FeCy₆ it is without action on the cystein anion.

Mn⁺⁺⁺ and Mn⁺⁺. If a few drops of manganous sulphate be added to cystein (in HCl solution) and the liquid made alkaline with ammonia a fine *green* coloration (Mn-cystein cpd.) appears. It rapidly fades provided sufficient cystein be present. On blowing air through the faded solution the colour is regenerated. The general behaviour of manganese towards cystein resembles that of iron described at length above.

It appears probable that the green body is the Mn⁺⁺⁺ derivative and the decolorised solution contains Mn⁺⁺. The addition of alkali to a manganous salt yields Mn(OH)₂, which however rapidly goes to Mn(OH)₃ or Mn₂O₃, H₂O

unless air be excluded. When cystein is also present the green colour is given in air, while in absence of air the solution remains colourless (Mn^{++}). When oxidation of cystein has reached completion brown Mn_2O_3 , H_2O remains. The addition of fresh cystein to this yields the green derivative and the sequence can then be repeated afresh.

Thunberg [1913] has found that in the presence of MnCl_2 the autoxidation of various thio-compounds is accelerated, a fact which is readily accounted for by a cycle such as the above, in which the salt functions as an oxygen carrier. According to Mathews and Walker [1909] however MnCl_2 has no effect on the velocity of oxidation of cystein.

Mn^{VI} . Dilute KMnO_4 added to cystein solution suffers decolorisation. On making alkaline with ammonia a *rose-red* colour appears, fading on standing and reappearing on aeration. The depth of colour is increased with increasing concentration of the two substances; excess of cystein producing more rapid fading on stoppage of the air current, coupled with a less readily regenerated colour on aeration, while increasing the amount of KMnO_4 gives a more stable coloration. Here again the behaviour is parallel with that of iron salts.

The rose colour may be due to sexvalent manganese, corresponding with MnO_3 , and the colourless reduced solution may contain quadrivalent Mn —(corresponding with MnO_2) formed from the MnO_3 by the reducing action of excess of cystein.

The first stage would be the production of MnO_2

In alkaline solution however Mn^{VI} is stable,

e.g.

hence we may have Mn^{VI} in the alkaline aerated cystein mixture. In the reduced solution MnO_2 is almost certainly present. The action of KMnO_4 is being further investigated.

Cu^{++} and Cu^+ . The existence of a copper derivative of cystein has been known for some time [Suter, 1895]. The addition of excess of a cupric salt to cystein in neutral solution produces a blue-black precipitate of a Cu^{++} -cystein compound. The latter is almost completely insoluble in neutral or slightly acid reaction, and may advantageously be employed for the separation and estimation of cystein [Emden, 1901]. When the solution is somewhat acid it is better to employ moist freshly precipitated $\text{Cu}(\text{OH})_2$ in place of the copper salt, following the procedure used by Hopkins [1921] in the separation of glutathione. The blue-black precipitate dissolves on addition of ammonia forming an intense dark brown (sepia) solution. It is reprecipitated on reducing the p_{H} to about 7 and dissolves to an almost colourless solution in excess of acid.

Interesting results have been obtained by the use of excess of cystein. The addition of cystein to the alkaline (brown) solution of the cystein-copper derivative causes rapid decolorisation. It will be shown that this is due to the reduction of the copper to the cuprous condition, and that the decolorised solution contains the cuprous-cystein derivative. If the decolorised solution be now shaken the dark brown colour reappears: the Cu^+ has been re-oxidised by the air in the alkaline solution to Cu^{++} . On standing, the solution fades once more; and these changes can be repeated so long as sufficient unoxidised cystein remains. By the prolonged action of oxygen or air on the brown solution the cystein is completely oxidised to cystine and a cuprammonium colour persists.

Copper is singular among the metals so far examined in that *both* its cystein salts (cuprous and cupric) are insoluble in neutral solution. As mentioned above an alkaline solution of the cuprous derivative is formed when the ammoniacal cupric—i.e. the brown—solution reduces spontaneously in the presence of excess of cystein. On neutralisation of the decolorised solution by the addition of dilute HCl the cuprous cystein compound is precipitated as a highly characteristic voluminous white precipitate. It may be obtained in a variety of ways. Add to a few cc. of a 5 % cystein solution one drop of CuSO_4aq . A blue-black precipitate of the cupric derivative first appears but is rapidly transformed into the white cuprous derivative. The Cu^{++} has become reduced to Cu^+ by excess of cystein. The white precipitate is readily soluble in excess of acid or ammonia to a colourless solution. The alkaline solution readily darkens in presence of air owing to oxidation of Cu^+ . The blackened alkaline solution will of course again yield the cupric cystein derivative on neutralisation, while the air-free clear alkaline solution will give the white precipitate.

The cuprous derivative has also been obtained directly by the use of cuprous iodide. To a cystein solution a little well washed freshly precipitated cuprous iodide was added and the solution made alkaline with ammonia. An almost colourless solution resulted. This was neutralised and yielded the characteristic white precipitate. The strongly alkaline solution readily darkens in air—owing to oxidation of the cuprous ion—especially when the amount of copper is large. The colour can be seen extending downwards from the exposed surface of a large tube.

Co. The addition of a drop of cobalt acetate to cystein solution followed by ammonia resulted in the production of a dark yellow colour. No appreciable intensification resulted on prolonged passage of air through the mixture. The intensity of colour was increased by addition of further Co solution.

Ni. Cystein solution containing a little NiSO_4 assumed an orange colour on addition of ammonia. The colour deepened to a reddish brown (mahogany) appearance when some more cystein and nickel sulphate were added. The colour was retained so long as cystein remained in the solution.

Cr. On adding cystein solution to a weak, acidified solution of $\text{K}_2\text{Cr}_2\text{O}_7$,

an intense chrome yellow colour appeared. On making alkaline with ammonia the usual greenish chromate colour was observed; it was not formed so readily however on the direct addition of ammoniacal $K_2Cr_2O_7$ to cystein. Potassium dichromate is peculiar in giving the coloration in *acid* solutions. Potassium chromate, which also contains the hexavalent chromium atom, behaves in the same way.

Sn^{iv}, Snⁱⁱ. The formation of a tin derivative of cystein may be demonstrated in the following manner. Have two test tubes each containing 3 cc. of water, and to one add also about 30 mg. of cystein. Add a little $SnCl_4$ to both tubes. Now make both tubes alkaline by the addition of ammonia and then acid by means of sulphuric acid. The presence of a tin cystein derivative is shown by its ready solubility in acid and more especially in ammoniacal solution. The tube containing the cystein will be seen to become perfectly clear and colourless in presence of ammonia or larger excess of acid; while a bulky precipitate remains in the blank tube under the same circumstances.

It might be thought that the presence of $SnCl_4$ would favour oxidation of the cystein. There seems no doubt however that the production of the clear solution is due to unoxidised cystein, since its presence is shown by the nitroprusside reaction. Indeed the presence of $SnCl_4$ seems to inhibit the oxidation. In one experiment a merest trace of cystein was added to a bottle containing a large excess of $SnCl_4$ and water, and the mixture was made alkaline with ammonia. It still showed a nitroprusside reaction after the lapse of five days.

Tin has a particularly strong affinity for sulphur and no doubt it protects that atom in the cystein molecule from oxidation.

With $SnCl_2$ the results observed were in every respect identical with those described above for $SnCl_4$.

Asⁱⁱⁱ, As^v. Arsenic appears to be too electro-negative to afford any ready evidence of functioning as the basic part of a cystein derivative. As_2S_2 and As_2S_3 were examined but without result.

Pb. The addition of $Pb(NO_3)_2$ to cystein solutions failed to yield a coloration.

Hgⁱⁱ. If insufficiency of "HgSO₄ reagent" is added to cystein the white precipitate first formed disappears. The same result can be obtained by addition of excess of cystein to the mercury precipitate. The phenomenon appears to be due to the reduction of the mercury from the bivalent to the univalent state, Hg' , which is inefficient as a precipitant. Analogous results may be obtained with other mercuric derivatives. If cystein be added in excess to a suspension (1) of *mercuric iodide*, or (2) of the *double ammonium compound* which is precipitated when ammonia is added to acidified mercuric iodide, the precipitate will be seen to dissolve in both cases.

When sufficient mercuric salt is added, however, precipitation occurs. Mercuric nitrate, chloride and acetate all yield insoluble compounds with cystein. These substances have no precipitant action on cystine.

Bi. Add cystein to an ammoniacal suspension of bismuth hydroxide (obtained from bismuth nitrate or subnitrate). The precipitate readily dissolves with the production of a yellow solution, indicative of a Bi^{+++} cystein compound.

The action of BiO_4 and Bi_2O_5 remains to be investigated.

It will be seen that the observations recorded above open up considerable ground for further inquiry. A number of metals have still to be investigated. In view of the fact that considerable supplies of cystein will be required for continuation of the research and in view of the calls of other research work it has been thought advisable to give an account of the observations so far made.

I should like to express my sincere gratitude to Professor Hopkins for the help and encouragement he has given me and the interest he has shown in this work.

SUMMARY.

(1) The so-called "ammonia" test for cystein is only effective in the presence of traces of a metallic compound.

(2) The following metals—viz. Fe^{+++} , Mn^{+++} , $\text{Mn}^{\text{v}^{(?)}}$, Cu^{++} , Hg^{++} —can form part of a system which is reversible so long as unoxidised cystein is present; the controlling factors being the relative concentrations of cystein, metal salt and atmospheric oxygen. In alkaline solution and in presence of oxygen, the reduced metal is equivalent to an oxygen acceptor, and in absence of oxygen the oxidised metal is equivalent to a donator of oxygen, the cystein forming the oxidisable body.

(3) The fading of alkaline solutions containing a metal *plus* cystein and the recoloration on aeration are explained on these grounds.

(4) Coloured metallic derivatives of cystein have been formed from Fe^{+++} , Mn^{+++} , $\text{Mn}^{\text{v}^{(?)}}$, Cu^{++} , Co, Ni, Cr, Bi. Cu^+ gives a characteristic white derivative insoluble in neutral solutions. Sn and Hg derivatives are also discussed.

(5) Cystein exhibits greater readiness to form metallic derivatives than is the case with cystine.

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