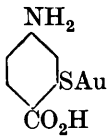
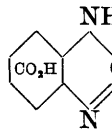
CCXL.—*Gold and Mercury Derivatives of 2-Thiolglyoxalines. Mechanism of the Oxidation of 2-Thiolglyoxalines to Glyoxalines.*

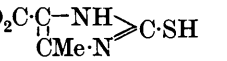
By Isidore Elkanah Balaban and Harold King.

The application of compounds of gold to the treatment of tuberculosis starts from an old observation by Koch that inhibition of the growth of tubercle bacilli by gold cyanogen compounds occurs at a dilution of 1 in 2 million, but the question as to the chemotherapeutic efficacy of organic gold compounds in tuberculosis is still an open one. Two organic compounds of gold have attained considerable prominence in Germany for the treatment of tuberculosis, namely, 4-amino-2-aurothiolbenzoic acid (krysolgan) (I) (D.R.-P. 349012) and 2-aurothiolbenziminazolecarboxylic acid (triphal) (II) (E.P. 225875 of 1923), but there is no record of the cure of experimental infections of tuberculosis in animals by these substances.

(I.) 

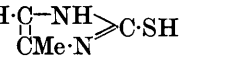
(II.) 

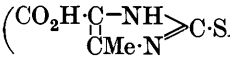
Both substances belong to a common type, of which an indefinite number could be prepared by synthesis, and the present communication is an attempt to determine whether there is any curative action, on experimental tuberculosis in animals, in this type of compound which would justify an extensive programme of syntheses in this field. For this purpose, ethyl 2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylate (III) has been chosen as a starting point, as it is fairly readily accessible from ethyl acetoacetate.

(III.) 

(IV.) 

When treated in methyl-alcoholic solution with auric chloride (1/3 mol.), it yields together with other products *ethyl 2-aurothiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylate* (IV), from which the carboxylic acid cannot be obtained without decomposition and separation of gold. The ester (III) can, however, be hydrolysed to *2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylic acid* (V), which under similar conditions yields *2-aurothiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylic acid* (VI).

(V.) 

(VI.) 

The action of auric chloride on mercaptans was first studied by Zeise (*Ann. Physik*, 1834, **31**, 369), who, from the analysis of the amorphous product, C_2H_5SAu, of the action of auric chloride on ethyl mercaptan, considered that two atoms of chlorine were set free which acted on another portion of the mercaptan. In agreement with this, Hermann (*Ber.*, 1905, **38**, 2813), who examined the action of auric chloride on benzyl and *iso*amyl mercaptans, found the optimum conditions to be given by the equation

$$AuCl_3 + 3R{\cdot}SH = R{\cdot}SAu + RS{\cdot}SR + 3HCl.$$

The auromercaptides were amorphous and the disulphides were definitely identified.

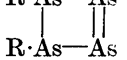
In the present instance, although the optimum conditions are determined by three molecular proportions of the thiolglyoxaline to one of auric chloride, the disulphide which should be produced in this reaction was never found, but only unchanged thiol acid. There is no doubt that the disulphide is initially produced, but, owing to the ease with which it acts as an acceptor for hydrogen, it is reduced during the subsequent operations necessary for its isolation. Attempts to make use of this ease of reversion of the disulphide to the thiol state, by using thiol acid and auric chloride in equimolecular proportion, to simplify the preparation of the 2-aurothiol acid (VI) which can be separated from the 2-thiol acid (V) only by a repetitive salting-out of the amorphous sodium salt of the aurothiol acid, were quite unsuccessful.

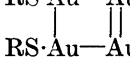
When auric chloride in methyl alcohol is added to the above-named thiolglyoxalines in methyl alcohol, there is invariably produced an intense blood-red coloration which persists for about 30 minutes and then fades to a pale yellow. There is no separation of gold. The same reaction is observed in aqueous hydrochloric acid solutions. It was first noted without comment by Gabriel and Pinkus (*Ber.*, 1893, **26**, 2203) for 2-thiol-4(or 5)-methylglyoxaline and by Tanret (*Compt. rend.*, 1909, **149**, 222) for ergothioneine, shown by Barger and Ewins (J., 1911, **99**, 2336) to be identical with 2-thiolhistidinebetaine. It is not, however, given by cysteine, thiocarbamide, allylthiocarbamide, or *p*-thiolphenylethylamine. We are not able to adduce any conclusive evidence as to the nature of this blood-red coloration. Whilst all the 2-thiolglyoxalines examined give the reaction instantaneously, 4(or 5)-*carbethoxy*-5(or 4)-*methylglyoxaline*-2-*disulphide* in methyl-alcoholic solution does not give it at once, but the colour gradually develops, lasts for many hours, and finally disappears. In hydrochloric acid solution the disulphide remains yellow for a few seconds on addition of chloroauric acid and then gives an amorphous, red precipitate

which becomes pale yellow after a few hours. One possibility is that the colour is due to the chromophoric influence of the sulphur atom on intermediate addition compounds of auric chloride with the thiolglyoxalines which gradually disappear as the auric chloride is used up in its reactions with the thiol group. This is supported by the observations of Wohl and Marckwald (*Ber.*, 1889, **22**, 1355), who found that platinum and gold chlorides formed with 2-thiol-1-methylglyoxaline brick-red and deep purple-red crystalline addition compounds, respectively, of the types, 2 base + $PtCl_4$, and base + $AuCl_3$, and of the present authors that the salt of 2-*ethylthiol*-4(or 5)-*methylglyoxaline* with chloroauric acid is red, whereas the addition compound of 2-phenyl-1-methylglyoxaline and auric chloride of the type base + $AuCl_3$ is deep yellow (J., 1926, 589). The other possibility is that the blood-red coloration is due to the transient formation of the free aurous radicals, R·SAu<, which polymerise to form the pale yellow auromercaptides R·SAu:AuS·R or, in general, $(R{\cdot}S{\cdot}Au{<})_n$. The properties of these auromercaptides are in agreement with this formulation : they are all amorphous substances with well-marked colloidal properties.

In the reaction of only one other reducing agent with auric solutions have we observed a transient red coloration. In the preparation of sodium gold thiosulphate, $Na_3Au(S_2O_3)_2$, by the action of a neutral solution of gold chloride on sodium thiosulphate, a transient red colour is obtained which, examined spectroscopically, has a similar general absorption in the visible region to the blood-red colour obtained with thiolglyoxalines. In this case also, the reaction is essentially a reduction to the aurous state. Brown's view (*J. Amer. Chem. Soc.*, 1927, **49**, 959) that the colour is due to the formation of sodium aurate does not tally with the colour of alkali aurates, which are pale yellow or pale green.

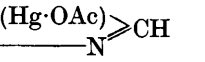
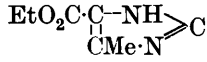
2-Aurothiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylic acid (VI) prepared as described above invariably contains an excess of combined gold in the approximate ratio of N:Au = 2 : 1·08 which finds a ready interpretation by analogy with the arsenicals. Just as over-reduction of arsinic acids leads to production of polyarsenides, which are usually formulated as in (VII), so here the product probably contains (VIII).

(VII.)


(VIII.)


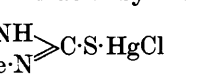
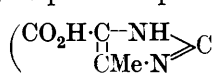
The reaction of thiolglyoxalines with mercuric salts is in striking contrast to their behaviour towards auric chloride. When ethyl glyoxaline-4(or 5)-carboxylate is digested in alcoholic solution with mercuric acetate, it yields *ethyl* 1-*acetoxymercuriglyoxaline*-4(or 5)-

carboxylate (IX) quantitatively, but ethyl 2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylate and the corresponding carboxylic acid, when digested with excess of mercuric chloride, yield *ethyl*

(IX.)   (X.)

2-*chloromercurithiol*-4(or 5)-*methylglyoxaline*-5(or 4)-*carboxylate* (X) and 2-*chloromercurithiol*-4(or 5)-*methylglyoxaline*-5(or 4)-*carboxylic acid* (XI) respectively. These are crystalline compounds and their constitution follows from their behaviour towards sodium hydroxide and ammonium sulphide.

Results of considerable interest have been obtained in an attempt to prepare the disulphide (XII) which should have arisen in the action of auric chloride on 2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylic acid (V). It cannot be obtained by oxidation of the corresponding thiol acid by iodine, as partial replacement of the

(XI.)   (XII.)

carboxyl group by iodine takes place, and it is not formed when 4(or 5)-*carbethoxy*-5(or 4)-*methylglyoxaline*-2-*disulphide* (XIII), prepared by the action of iodine upon ethyl 2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylate, is hydrolysed by alkalis or acids. After this ester disulphide (XIII) had been boiled for an hour with 10% aqueous sodium carbonate, the products isolated were 2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylic acid (V), the corresponding decarboxylated product 2-thiol-4(or 5)-methylglyoxaline, ethyl 2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylate (III), and sulphur dioxide. On acid hydrolysis, 2-thiol-4(or 5)-methylglyoxaline was also obtained with other substances. Quantitative estimation of the sulphur dioxide produced showed that 74% of that required by the equation $2RS{\cdot}SR + 2H_2O = 3R{\cdot}SH + RH + SO_2$ was present. The mother-liquor contained sulphate, so that the deficiency in sulphur dioxide is probably accounted for by the reducing action of sodium sulphite on the disulphide, for the disulphide (XIII) is readily reduced to the thiol ester in aqueous solution by sulphur dioxide.

It seems probable that an unstable sulphinic acid is the intermediate product in the above hydrolysis, in which case the reaction would be the reverse of the mode of synthesis of disulphides of Otto and Schiller (*Ber.*, 1876, **9**, 1589):

$$3R{\cdot}SH + R{\cdot}SO_2H = 2RS{\cdot}SR + 2H_2O.$$

In support of this view, the first sulphinic acid in the glyoxaline series has been prepared and proves to be a very unstable substance

readily yielding sulphur dioxide at room temperature. When 2-thiol-4(or 5)-methylglyoxaline (XIV) is added at 0° to 10% hydrogen peroxide (2 mols.), it is converted for the most part into 4(or 5)-*methylglyoxaline-2-sulphinic acid* (XV) with simultaneous formation of small quantities of 4(or 5)-*methylglyoxaline-2-sulphonic acid* (XVI), 4(or 5)-methylglyoxaline, and sulphuric acid.

(XIII.) $\left(\begin{array}{c}EtO_2C\cdot C\!-\!NH\\ \|\quad\quad\rangle C\cdot S-\\ CMe\cdot N\end{array}\right)_2$ $\begin{array}{c}CMe\cdot NH\\ \|\quad\rangle C\cdot SH\\ CH\!-\!N\end{array}$ (XIV.)

(XV.) $\begin{array}{c}CMe\cdot NH\\ \|\quad\rangle C\cdot SO_2H\\ CH\!-\!N\end{array}$ $\begin{array}{c}CMe\cdot NH\\ \|\quad\rangle C\cdot SO_3H\\ CH\!-\!N\end{array}$ (XVI.)

The sulphinic acid is a crystalline solid, stable at 0°. It has an acid reaction, but at room temperature it changes after a few hours into a strongly basic liquid containing 4(or 5)-methylglyoxaline and its *sulphite*. It consumes one atom of oxygen when titrated with permanganate at 0°, and is converted by excess of hydrogen peroxide, preferably in the presence of alkali, into the sulphonic acid. The formation of this unstable sulphinic acid is, in fact, exactly analogous to the formation of the somewhat more stable aminoiminomethanesulphinic acid, $NH{:}C(NH_2)\cdot SO_2H$, by the oxidation at 0° of thiocarbamide by hydrogen peroxide (Barnett, J., 1910, **97**, 63).

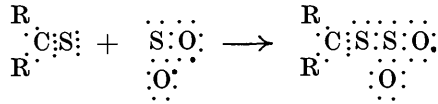
The instability of the glyoxalinesulphinic acid affords a satisfactory interpretation of the desulphuration of 2-thiolglyoxalines by means of nitric acid discovered by Wohl and Marckwald (*Ber.*, 1889, **22**, 575). These authors postulated the formation of an unstable sulphonic acid which was hydrolysed even under the conditions of oxidation :

$$N{:}C(SH)\cdot NH^- \longrightarrow {-}N{:}C(SO_3H)\cdot NH^- \longrightarrow {-}N{:}CH\cdot NH^- + H_2SO_4.$$

This explanation was, however, disproved by the observations of Anschütz and Schwickerath (*Annalen*, 1895, **284**, 9), Biltz and Krebs (*ibid.*, 1912, **391**, 203), and Lamb and Pyman (J., 1924, **125**, 707). Biltz and Krebs favoured the view that disulphides are the intermediate products, but the results are much more compatible with the view that an unstable sulphinic acid is formed, and the more unstable it is, the smaller the proportion of sulphonic acid formed in the oxidation and the larger the proportion of desulphurised glyoxaline.

When the alkaline hydrolysate of 4(or 5)-carbethoxy-5(or 4)-methylglyoxaline-2-disulphide (XIII) is acidified to Congo-paper, the solution turns yellow and evolves sulphur dioxide. On removal of this by aspiration, the yellow colour vanishes, but it is restored when sulphur dioxide is passed into the solution. This has been traced to the formation of unstable, yellow or orange-yellow addition

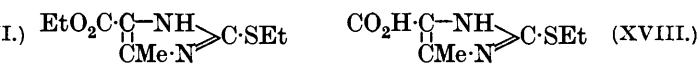
compounds between many 2-thiolglyoxalines and sulphur dioxide in aqueous or acid solution or in the dry state. Quantitative measurement of the amount of sulphur dioxide absorbed by dry 2-thiol-1-phenylglyoxaline showed that exactly one molecule is absorbed under one atmosphere of sulphur dioxide, but on exposure to the air the compound has a half-life period of 10 minutes. The reaction is also given by thiocarbamide, and is strikingly shown by allylthiocarbamide and 2-thiol-1 : 4-dimethylglyoxaline, which are immediately converted into yellow and orange-yellow liquids, respectively, by exposure to sulphur dioxide. The constitution of these labile addition compounds is best interpreted as a simple addition of the sulphur atom of the thiol group to the electromeric modification of sulphur dioxide containing two semi-polar double bonds.



This interpretation does not discriminate between the –NH·C(SH):N– structure and the –NH·CS·NH– structure of 2-thiolglyoxalines. Korczyński and Glebocka (*Gazzetta*, 1920, **50**, i, 378) have described a number of addition compounds of sulphur dioxide with a variety of amines, including thiocarbamide, and attribute their formation to the secondary valencies of the nitrogen atom. Ethyl 4(or 5)-glyoxalinecarboxylate, however, does not add on sulphur dioxide, so that their interpretation does not hold here.

The similarity between thiocarbamides and 2-thiolglyoxalines in their behaviour towards sulphur dioxide extends to the colour reaction described by Sato (*Biochem. Z.*, 1909, **23**, 45) for thiocarbamides, and an improved form of the test is described in the experimental portion together with a number of other colour reactions which may prove of service in this field.

In order to characterise 2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylic acid (V) more fully, it has been converted by esterification by alcoholic hydrogen chloride (compare Burtles, Pyman, and Roylance, J., 1925, **127**, 588) into *ethyl* 2-*ethylthiol*-4(or 5)-*methylglyoxaline*-5(or 4)-*carboxylate* (XVII), which on hydrolysis yields 2-*ethylthiol*-4(or 5)-*methylglyoxaline*-5(or 4)-*carboxylic acid* (XVIII) and its decarboxylation product, 2-*ethylthiol*-4(or 5)-*methylglyoxaline*.



The acid (XVIII) forms a *monohydrochloride* and a *semihydrochloride* and thus resembles some of the amino-acids, such as glycine and *dl*-alanine, which form normal and semi-hydrochlorides,

and the many anomalous amine salts recorded by Werner (*Ber.*, 1903, **36**, 149). The majority of these were formulated by Werner in terms of co-ordinated hydrogen: $XH + 2NR_3 = X{\cdot}H\begin{matrix}\diagup NR_3\\ \diagdown NR_3\end{matrix}$, which on the electronic theory of valency would be readily interpreted as

$$XH + 2R{:}\underset{\cdot\cdot}{\overset{\cdot\cdot}{\overset{R}{\underset{R}{N}}}}{:} = \left[R{:}\underset{\cdot\cdot}{\overset{\cdot\cdot}{\overset{R}{\underset{R}{N}}}}{:}H{:}\underset{\cdot\cdot}{\overset{\cdot\cdot}{\overset{R}{\underset{R}{N}}}}{:}R\right]^{+}\ X^{-}.$$

The case is in fact the parallel of the interpretation put forward for potassium hydrogen fluoride, KHF_2, where, however, co-ordinated hydrogen links the fluorine atoms in a negatively charged complex (Lewis, " Valence," 1923, 110). A difficulty, however, arises in the interpretation of the compounds $(NH_3)_4$,HCl (Joannis, *Compt. rend.*, 1902, **135**, 1106), $(NH_3)_4$,HBr (Bakhuis-Roozeboom, *Rec. trav. chim.*, 1885, **4**, 361), and $(NH_3)_4$,HNO_3 (Kuriloff, *Z. physikal. Chem.*, 1898, **25**, 108). These would indicate a co-ordination number of 4 for hydrogen or else chain formation of ammonia molecules with links of co-ordinated hydrogen.

We are indebted to Captain S. R. Douglas, F.R.S., for a determination of the effect of sodium aurothiolmethylglyoxalinecarboxylate on tubercle bacilli. It inhibits the growth of these organisms *in vitro* at a dilution of 1 in 100,000 (not tested at lower concentrations). Guinea-pigs were injected each with 0·5 mg. of a virulent culture of tubercle bacilli which had been subjected for 12 hours to a concentration of the sodium salt ranging from 1 in 1,000,000 to 1 in 1000. The time elapsing between injection and death of the animal from tuberculosis was progressively longer with increasing concentration of the salt used in the preliminary treatment of the bacilli. In no case had sterilisation been effected. Rabbits inoculated with a virulent strain were injected weekly with the sodium salt (0·01 g. per kilo.) and after four injections the lungs showed less signs of tubercular lesions than the controls, whilst the death of mice inoculated with the bacilli was distinctly delayed by one and by two injections of the sodium salt, 50 mice being used as controls, 25 mice for one dose and 25 for two doses.

To Miss Durham and Miss Marchal we are indebted for a determination of the toxicities to mice of sodium aurothiolmethylglyoxalinecarboxylate (0·05 mg. per g.) and of sodium 2-chloromercurithiolmethylglyoxalinecarboxylate (0·005 mg. per g.). The same compounds were tested for curative action on mice infected with trypanosomes, but no such action was detected.

Our thanks are also due to Professor F. L. Pyman, F.R.S., for the gift of samples of a series of thiolglyoxalines from his unique collection.

EXPERIMENTAL.

Hydrolysis of Ethyl 2-Thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylate (III).—This ester was prepared in 42% yield from ethyl acetoacetate as described by Posner and Gabriel (*Ber.*, 1894, **27**, 1141), but Wolff's method (*Annalen*, 1902, **325**, 135) was used for the preparation of oximinoacetoacetic ester. It melts at 236—237° (P. and G. give 229°) and is soluble in 64 parts of boiling water.

The ester (50 g.) was boiled for 1 hour with 100 g. of sodium carbonate (anhydrous) in 1000 c.c. of water, and the reaction then adjusted to neutrality to Congo-paper. The precipitate and the successive crops obtained by evaporation of the mother-liquors (in all, 38·3 g.) were extracted with one-half saturated sodium hydrogen carbonate solution, which removed the whole of the carboxylic acid and the major portion of the decarboxylated base, leaving behind non-hydrolysed ester mixed with a little decarboxylated base. The main alkaline extract on neutralisation to Congo-paper gave the carboxylic acid (23·35 g.), and the mother-liquors on concentration gave the thiol base (3·7 g.). The portion insoluble in sodium hydrogen carbonate solution (9·05 g.) was boiled with water (50 c.c.) and left, undissolved, almost pure ester (3·5 g.); the water-soluble fraction was made alkaline with sodium carbonate and boiled down in an open flask to hydrolyse the ester still present. From this alkaline liquor the thiol base was recovered in successive crops, 6·0 g. in all, the mother-liquors which gave the thiol base (3·7 g.) being incorporated at one stage. The final liquor was acidified to Congo-paper and gave a small crop of carboxylic acid (0·4 g.).

2-Thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylic acid (V) is soluble in 100 parts of boiling water and separates in granular crystals. It melts with loss of carbon dioxide at 240—241°, and the crystalline residue obtained, at 244—245°, the m. p. of 2-thiol-4(5)-methylglyoxaline (Found: S, 20·5; N, 17·9. $C_5H_6O_2N_2S$ requires S, 20·3; N, 17·2%). The acid gives an immediate red colour with Pauly's reagent in sodium carbonate solution.

Esterification of 2-Thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylic Acid.—The acid (5 g.) was suspended in 100 c.c. of absolute alcohol, and the liquid saturated with hydrogen chloride at 0°. The suspension was then boiled for 3 hours with further passage of the gas. After removal of alcohol and excess of acid by evaporation with water, the syrupy residue was dissolved in 20 c.c. of water and treated with saturated potassium carbonate solution so long as a crystalline precipitate separated. This amounted to 4·4 g., and

when crystallised from the minimum volume (350 c.c.) of boiling water separated in long, white, silky needles (4·05 g.), m. p. 144—145°.

Ethyl 2-*ethylthiol*-4(or 5)-*methylglyoxaline*-5(or 4)-*carboxylate* (XVII) is readily soluble in boiling organic solvents, but crystallises conveniently only from acetone, ethyl acetate, or water (Found in air-dried material : loss at 100°, 10·7. $C_7H_{10}O_2N_2S,1\frac{1}{2}H_2O$ requires H_2O, 11·2%. Found in anhydrous solid : N, 12·8. $C_7H_{10}O_2N_2S$ requires N, 13·1%). It forms very soluble salts with nitric, hydrochloric and sulphuric acids, the *hydrochloride* crystallising in needles. It forms an oily *chloroaurate*, but a *picrate* crystallising in glistening, yellow prisms, m. p. 135—136° (Found : picric acid by nitron, 52·1. $C_9H_{14}O_2N_2S,C_6H_3O_7N_3$ requires picric acid, 51·7%).

In a preliminary experiment (1·6 g. of thiol acid) where esterification was only carried out for 90 minutes, the product precipitated at neutrality (1·35 g.) gave by ether extraction 1·25 g. of *S*-ethyl ether and 0·1 g. of insoluble material which proved to be ethyl 2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylate.

S-*Alkylation of Ethyl* 2-*Thiol*-4(or 5)-*methylglyoxaline*-5(or 4)-*carboxylate*.—The 2-thiolcarboxylic ester (10 g.) was suspended in 100 c.c. of absolute alcohol and submitted to the same treatment as in the foregoing esterification. The yield of crude *S*-ethyl ether was 10·8 g. It was recrystallised from 100 volumes of boiling water and gave 10·3 g. of pure material crystallising in needles, m. p. 144—145°. On prolonged standing, the mother-liquor deposited stout, triangular plates which proved to be the same substance. m. p. 143—144°.

Alkaline Hydrolysis of Ethyl 2-*Ethylthiol*-4(or 5)-*methylglyoxaline*-5(or 4)-*carboxylate*.—The ethyl ester of the *S*-ethyl ether (8·1 g.) was boiled for 4 hours with 16·2 g. of anhydrous sodium carbonate in 162 c.c. of water. The ethereal extract on evaporation gave 2·3 g. of 2-*ethylthiol*-4(or 5)-*methylglyoxaline*, m. p. 68—69°. This base was insoluble in light petroleum and very soluble in benzene, and crystallised well from ethyl ether in delicate, rectangular leaflets, m. p. 69—71°. It was converted into the *picrate* (5·5 g.), which was soluble to the extent of 2·3% in boiling water and separated on cooling in prismatic needles, m. p. 136—137° (Found : picric acid by nitron, 62·6, 62·7. $C_6H_{10}N_2S,C_6H_3O_7N_3$ requires picric acid, 61·7%). The picrate was converted into the hydrochloride, which could not be crystallised, and a portion of the latter precipitated with chloroauric acid. The *chloroaurate* separated in red plates, m. p. 130—131° without decomposition (Found : Au, 41·0. $C_6H_{10}N_2S,HAuCl_4$ requires Au, 40·9%).

The alkaline liquors after ethereal extraction were made very

faintly acid to Congo-paper and concentrated; two crops of needles, 4·05 g. in all, were then obtained. Of this, 3·3 g. were dissolved in 10 c.c. of boiling water in a covered vessel to avoid access of nuclei from the air. When the solution was cool and supersaturated, it was inoculated with nuclei of the needles. The crop of needles (2·3 g.) was collected and washed with ice-cold water [Found: Cl, 8·3; N, 13·4. $(C_7H_{10}O_2N_2S)_2,HCl$ requires Cl, 8·7; N, 13·7%]. This *semihydrochloride* of 2-*ethylthiol*-4(or 5)-*methylglyoxaline*-5(or 4)-*carboxylic acid* was very readily soluble in water, with a reaction faintly acid to Congo-paper. It melted rather indefinitely with effervescence between 122° and 125°, but without loss of carbon dioxide, as the residue did not give a picrate. If a strong aqueous solution was heated, the free *carboxylic acid* (XVIII) separated in stout rhombs; on cooling, needles of the semi-hydrochloride invariably separated, but the rhombs, even on prolonged standing, remained unchanged. If alkali was added until the faint acidity to Congo-paper was completely removed and the reaction was very faintly acid to sensitive methyl-orange paper, the free carboxylic acid was precipitated. It was soluble in 10 parts of boiling water and crystallised in large, rectangular tablets, m. p. 179—180° (efferv.) (Found: N, 14·9. $C_7H_{10}O_2N_2S$ requires N, 15·0%). The decarboxylated melt solidified and melted at 67° and gave a picrate identical with 2-ethylthiol-4(or 5)-methylglyoxaline picrate.

If the semi-hydrochloride (0·2 g.) be dissolved in a few drops of hot 3*N*-hydrochloric acid it forms a syrupy solution which soon deposits large, triangular prisms of the *monohydrochloride*, m. p. 189—190° (efferv.) (Found: Loss at 100°, 7·8. $C_7H_{10}O_2N_2S,HCl,H_2O$ requires H_2O, 7·5%. Found in dried salt: Cl, 16·0. $C_7H_{10}O_2N_2S,HCl$ requires Cl, 15·9%). This salt is very soluble in water, the solution turning Congo-paper blue.

Preparation of 5(or 4)-*Carbethoxy*-4-(or 5)-*methyl*-2-*glyoxaline Disulphide* (XIII).—Ethyl 2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylate (1 g.) was dissolved in 1000 c.c. of hot water, cooled to 50°, and treated with *N*/10-iodine solution until a slight excess was present as shown by the pale brown colour. Usually 55 c.c. were required instead of the theoretical 53·7 c.c. Three further batches were treated similarly. The *disulphide* (3·25 g.) which separated during the addition of iodine was collected; the mother-liquors, on being again treated with iodine until present in slight excess, and then neutralised with sodium carbonate, deposited a further 0·2 g. of disulphide. The product (yield, 86%), m. p. 220—222°, separated from boiling alcohol (120 c.c.) as a voluminous felt of pale yellow needles; after a further crystallisation, the m. p. was con-

stant at 222—223° (yield, 3·25 g.) (Found : N, 15·3. $C_{14}H_{18}O_4N_4S_2$ requires N, 15·1%). The same ester with the same m. p. was obtained by oxidation of the thiol ester in alkaline solution with potassium ferricyanide, but it was difficult to free it from Prussian blue, formed in traces. It was also obtained by dissolving the thiol ester in spirit, adding a lump of fused sodium nitrite, and running *N*-sulphuric acid on to the nitrite so that there was a vigorous evolution of gases through the liquid. The solution eventually deposited the disulphide. A modification of this method may prove to be the most accessible route to this disulphide. In sodium carbonate solution, the disulphide gives a yellow colour with Pauly's reagent. The ester is readily reduced to the thiol ester by passage of sulphur dioxide in aqueous solution.

Hydrolysis of the Disulphide.—(a) *By alkali.* The disulphide (7·4 g.) was boiled for 1 hour with 15 g. of anhydrous sodium carbonate in 150 c.c. of water. The solution, on being neutralised to Congo-paper, turned yellow, evolved sulphur dioxide, and gave a fraction (2·95 g.). On concentration, two further fractions (0·45 and 1·6 g.) were obtained. The sodium hydrogen carbonate extracts of these fractions gave, on neutralisation to Congo-paper, 2·76 g. of 2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylic acid, identical in all its reactions with the product of hydrolysis of the corresponding 2-thiol ester. The portion soluble in sodium hydrogen carbonate but not precipitated on neutralisation was isolated by concentration and proved to be pure 2-thiol-4(or 5)-methylglyoxaline (0·1 g.), m. p. 244—245°. The portion of original material insoluble in sodium hydrogen carbonate, on three crystallisations from water, gave 0·4 g. of ethyl 2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylate, m. p. 237°.

Quantitative determinations of the proportion of sulphur dioxide formed in the hydrolysis of the disulphide were made by hydrolysing 1·23 g. (*M*/300) with 2·46 g. of anhydrous sodium carbonate in 24·6 c.c. of water for 1 hour at 100°. The alcoholic distillate contained only a trace of reducing substance, possibly acetaldehyde. The alkaline mother-liquor was made acid by addition of 3*N*-hydrochloric acid, and the liberated sulphur dioxide absorbed by aspiration into standard *N*/10-iodine solution, any volatile iodine being caught in *N*/10-sodium thiosulphate solution. In two separate experiments, 72·4 and 73·5% of the sulphur dioxide required by the equation $2RS{\cdot}SR + 2H_2O = SO_2 + RH + 3RSH$ was found. The mother-liquors also contained appreciable amounts of sulphate. The method of estimation was controlled by experiments on standard sodium sulphite solution and found to agree within 2%.

(b) *By acid.* The disulphide (0·9 g.) was boiled for 3 hours with

10 c.c. of 16% hydrochloric acid. The solution was evaporated dry at 50°, and the residue was dissolved in a little water and made faintly alkaline to litmus; an amorphous base (0·4 g.) then separated. This was probably a mixture of bases, as it did not behave normally on reduction to the thiol base with sulphur dioxide or on oxidation to a base free from sulphur by dilute nitric acid. After removal of the amorphous substance, the mother-liquors, on concentration, gave 0·2 g. of crude 2-thiol-4(or 5)-methylglyoxaline, m. p. 239°. Recrystallised from 1·2 c.c. of hot water, it separated in the characteristic large crystals of the pure thiol base, m. p. 244°.

Preparation of Ethyl 2-Aurothiol-4(or 5)-*methylglyoxaline-5*(or 4)-*carboxylate* (IV).—Ethyl 2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylate (1·86 g.; 3 mols.) in 100 c.c. of dry methyl alcohol was treated with 1 g. (1 mol.) of gold trichloride in 30 c.c. of methyl alcohol. The solution became brilliant ruby-red and after 2 hours was pale yellow. The alcohol was removed and treatment of the residue with 400 c.c. of boiling water left 0·22 g., m. p. 254° (decomp.), undissolved. On keeping, the solution deposited needles (1·35 g.), m. p. 170°, containing 30·3% of combined gold. These were extracted with a further 150 c.c. of boiling water, which left 0·35 g. of solid undissolved, m. p. 252° (decomp.). This was the required *ethyl 2-aurothiol-4*(or 5)-*methylglyoxaline-5*(or 4)-*carboxylate* (Found: Au, 50·6. $C_7H_9O_2N_2SAu$ requires Au, 51·6%). Attempts to convert this ester into the corresponding acid by hydrolysis led to decomposition with separation of gold.

Preparation of *2-Aurothiol-4*(or 5)-*methylglyoxaline-5*(or 4)-*carboxylic Acid* (VI).—2-Thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylic acid (4·7 g.), dissolved in 435 c.c. of pure dry methyl alcohol, was treated with 3·0 g. (1/3 mol.) of gold trichloride in 10 c.c. of methyl alcohol. After 1 hour, the deep ruby-red colour which developed immediately on mixing had completely gone. The yellow solution was concentrated to about 10 c.c. and diluted with 250 c.c. of water. The solution set to a jelly which rapidly broke up with deposition of a mixture of amorphous and crystalline matter. There was no free gold present. The solid was collected, and dissolved in 100 c.c. of water with the aid of 22 c.c. of saturated sodium hydrogen carbonate solution. Addition of 30 g. of sodium chloride caused the separation of a white, amorphous precipitate of the sodium salt of the crude aurothiol acid. It was redissolved in 100 c.c. of water, by gentle warming with addition of a few drops of 2*N*-sodium hydroxide, and reprecipitated by 30 g. of sodium chloride. This process was repeated in order to eliminate the last traces of thiol acid. The amorphous sodium salt was suspended in water and treated with excess of 3*N*-hydrochloric acid, and the

amorphous aurothiol acid was centrifuged off, resuspended in water, and re-centrifuged so long as the supernatant fluid showed chloridion. The acid, after being dried in a vacuum and ground, was obtained as a pale primrose-yellow powder (yield, 2·7 g. or 77%) (Found : Loss on prolonged drying at 110°, 6·3%. Found in anhydrous solid : N, 7·6; Au, 57·8, 57·8. $C_5H_5O_2N_2SAu$ requires N, 7·9; Au, 55·6%). On ignition even with nitric and sulphuric acids, this aurothiol acid leaves a residue of gold soluble in cold aqua regia but containing a small quantity of insoluble black material. This is destroyed by digestion with hot aqua regia, and after removal of nitric acid the gold can be quantitatively precipitated by excess of ferrous chloride in acid solution. If insufficient acid be added, the gold contains a large quantity of iron (compare Treadwell, " Analytische Chemie," 1921, II, 213).

The aurothiol acid forms a clear pale yellow solution on neutralisation with sodium hydrogen carbonate and can be boiled without decomposition.

The first two sodium chloride precipitation liquors of the sodium salt, on acidification to Congo-paper, gave precipitates of 1·37 g. and 0·1 g., respectively, of crystalline 2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylic acid. This was identical in all its reactions with the 2-thiol acid prepared by hydrolysis of its ethyl ester. In particular, 1·2 g. were esterified by alcoholic hydrogen chloride and gave 1·1 g. of ethyl 2-ethylthiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylate.

In some experiments, the salting-out of the amorphous sodium salt of the aurothiol acid led to simultaneous separation of flattened needles of the sodium salt of the 2-thiol acid. This could be eliminated only by increasing the number of precipitations.

Action of Mercuric Acetate on Ethyl Glyoxaline-4(or 5)-*carboxylate.* —Ethyl glyoxalinecarboxylate (0·7 g.) was dissolved in 25 c.c. of alcohol and treated with 1·6 g. (1 mol.) of mercuric acetate. The gelatinous mass immediately produced was boiled for 6 hours; it then consisted of homogeneous, microscopic, flat prisms almost insoluble in cold alcohol (yield, 2·0 g.). *Ethyl* 1-*acetoxymercuri-glyoxaline*-4(5 ?)-*carboxylate* (IX) is instantly soluble in dilute hydrochloric acid, and black mercuric sulphide is precipitated from the solution by hydrogen sulphide. Sodium hydroxide produces orange-yellow mercuric oxide. The ester has no definite melting point, but shrinks suddenly at about 222° and is not further changed at 300° (Found : Loss at 100°, 2·8. $C_8H_{10}O_4N_2Hg,\frac{1}{2}H_2O$ requires H_2O, 2·2%. Found in dried substance : N, 6·8; Hg, 50·8. $C_8H_{10}O_4N_2Hg$ requires N, 7·0; Hg, 50·4%).

Action of Mercuric Chloride on Ethyl 2-*Thiol*-4(or 5)-*methyl-glyoxaline*-5(or 4)-*carboxylate.*—The thiol ester (1·86 g.) was boiled

with 5·4 g. (2 mols.) of mercuric chloride in 50 c.c. of absolute alcohol for 6 hours. On standing for a few days, *ethyl 2-chloromercurithiol*-4(or 5)-*methylglyoxaline*-5(or 4)-*carboxylate* (X), m. p. 167—168°, separated in six-sided plates (yield, 95%). It was soluble without difficulty in 3 volumes of boiling absolute alcohol (Found: N, 6·5. $C_7H_9O_2N_2ClSHg$ requires N, 6·7%). The ester does not give mercuric oxide on treatment with sodium hydroxide, but is immediately blackened by ammonium sulphide.

Action of Mercuric Chloride on 2-Thiol-4(or 5)-*methylglyoxaline*-5(or 4)-*carboxylic Acid.*—The thiol acid (1·58 g.), dissolved in 100 c.c. of pure dry methyl alcohol, was boiled for 6 hours with 5·43 g. (2 mols.) of mercuric chloride. After remaining for 12 hours at room temperature, the solution deposited 0·25 g. of substance, and on concentration a further 0·8 g. *2-Chloromercurithiol*-4(or 5)-*methylglyoxaline*-5(or 4)-*carboxylic acid* (XI), m. p. 257° (efferv.), is readily soluble in sodium hydrogen carbonate solution and is precipitated unchanged, but microcrystalline, on addition of hydrochloric acid (Found: N, 7·1. $C_5H_5O_2N_2ClSHg$ requires N, 7·1%). Mercuric sulphide is readily precipitated when hydrogen sulphide is passed into a suspension of the acid in hot dilute mineral acid solution, or instantly by the action of ammonium sulphide. The acid does not give mercuric oxide on treatment with sodium hydroxide.

Action of Hydrogen Peroxide on 2-Thiol-4(or 5)-*methylglyoxaline.*—Finely powdered thiolmethylglyoxaline (1·14 g.) was added in portions to a stirred solution of 2·4 g. (2 mols.) of Merck's perhydrol in 6 c.c. of water at 0° to −5°. The thiol compound rapidly passed into solution through oxidation and yielded a clear solution which after a short time became filled with microscopic leaflets of 4(or 5)-*methylglyoxaline-2-sulphinic acid* (XV). These were filtered off on an ice-jacketed funnel at 0° and washed with ice-cold water (yield, about 0·7 g.). For analysis, portions were removed, rubbed quickly on ice-cold porous plate, and rapidly weighed (Found: N, by Kjeldahl's method, 15·7; SO_2 evolved by digestion with boiling dilute sulphuric acid and absorbed by aspiration in *N*/10-iodine, 36·7; N in distillation residue, by Kjeldahl's method, 15·9—whence $SO_2 : N = 1·01 : 2$. $C_4H_6O_2N_2S,2H_2O$ requires SO_2, 35·2; N, 15·4%. Found: oxygen consumed by oxidation with *N*/10-permanganate in 2*N*-sulphuric acid at 0°, 8·1. $C_4H_6O_2N_2S,2H_2O$ requires O, 8·8%). This sulphinic acid is an unstable substance. When kept for a few hours either in a vacuum over sulphuric acid or in the air at room temperature, it passes into a clear basic liquid with loss of some sulphur dioxide. This liquid residue, when kept dry or when heated on the water-bath, partly crystallises as the normal *sulphite* of 4-methylglyoxaline. If the basic residue be

mixed with saturated potassium carbonate solution and extracted with chloroform, the latter on evaporation yields a base which readily crystallises completely as 4-methylglyoxaline. The sulphinic acid melts at 73° and effervesces at 92°. It is faintly acid to sensitive methyl-orange paper and dissolves in sodium hydrogen carbonate solution with evolution of carbon dioxide. It is not very readily soluble in ice-cold water and gives no precipitate with ferric chloride. Its ammoniacal solution gives no precipitate with calcium or barium chloride.

The mother-liquors of the oxidation were evaporated to a small volume over sulphuric acid in a desiccator and then deposited clusters of fine needles (0·1 g.) of the corresponding sulphonic acid (see below). The mother-liquor also contained sulphate and 4-methylglyoxaline, the latter being readily removed from saturated potassium carbonate solution by chloroform.

4(or 5)-*Methylglyoxaline-2-sulphonic Acid* (XVI).—2-Thiol-4(or 5)-methylglyoxaline (0·57 g.) was oxidised by hydrogen peroxide (3 mols.) in the way described in the previous section. When the sulphinic acid had crystallised from the liquor, 2*N*-sodium hydroxide (2 c.c.) was added drop by drop until an alkaline reaction was attained. The clear solution was concentrated at room temperature over sulphuric acid in a vacuum for 12 hours and had then become acid (decomposition of sulphinic acid) in reaction with deposition of a few crystals of the sulphonic acid. It was made acid to Congo-paper and gradually deposited the sulphonic acid (yield 0·55 g.). For analysis, 0·35 g. was dissolved in 0·2 c.c. of hot water and gave 0·3 g., crystallising in stout columns, m. p. about 280° (Found : Loss at 100°, 10·3. $C_4H_6O_3N_2S,H_2O$ requires H_2O, 10·0%. Found in dried substance : N, 16·9. $C_4H_6O_3N_2S$ requires N, 17·2%). This sulphonic acid is faintly acid to Congo-paper and methyl-orange paper and is stable to acid permanganate. The *calcium* and *barium* salts are very soluble in water. The *ammonium* salt is readily soluble, but crystallises well in microscopic rods.

Action of Sulphur Dioxide on 2-Thiolglyoxalines.—If 2-thiol-4(or 5)-methylglyoxaline, ethyl 2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylate or 2-thiol-4(or 5)-methylglyoxaline-5(or 4)-carboxylic acid be dissolved in *N*-hydrochloric acid and subjected to a current of sulphur dioxide, the solution immediately becomes yellow. The same behaviour is shown by thiocarbamide but not by cysteine. When 2-thiol-1-phenylglyoxaline, 2-thiol-1-phenyl-4-methylglyoxaline, 2-thiol-1 : 5-dimethylglyoxaline, and 2-thiol-1 : 4-dimethylglyoxaline are dissolved in water and saturated with sulphur dioxide, yellow solutions are produced and in the case of the two 1-phenyl derivatives orange-yellow crystalline addition

products separate readily. These are stable in presence of excess of sulphur dioxide, but redissolve as the sulphur dioxide passes off. 2-Thiol-4 : 5-diphenylglyoxaline, unlike all the other 2-thiolglyoxalines examined, showed no visible change, possibly owing to its sparing solubility in water. When sulphur dioxide is passed over powdered 2-thiolglyoxalines, they become yellow to orange-yellow through absorption of the gas. Thus 2-thiol-1-phenyl-, 2-thiol-1-phenyl-4-methyl-, 2-thiol-1 : 5-dimethyl-, 2-thiol-4-methyl-, and 5-carbethoxy-2-thiol-4-methyl-glyoxalines all become yellow on exposure to sulphur dioxide. Thiocarbamide became pale yellow, but 2-thiol-1 : 4-dimethylglyoxaline and allylthiocarbamide (thiosinamine) immediately liquefied on exposure to the gas, forming orange and yellow liquids respectively. 5-Carbethoxy-4-methyl-2-glyoxaline disulphide went deeper yellow on exposure to the gas and in aqueous suspension was rapidly reduced to the corresponding thiol compound. The hydrated form of ergothioneine from blood was immediately converted into an orange-coloured, pasty product by exposure to sulphur dioxide.

Quantitative Absorption Experiments.—Finely divided 2-thiol-1-phenylglyoxaline (0·0712 g.) was submitted to a current of sulphur dioxide in a small U-tube fitted with pierced ground glass stoppers and side arms. Absorption of the gas was rapid and at the end of 2 hours 0·0249 g. had been absorbed. From this point absorption was slow and after 12 hours the total absorption was 0·0263 g., corresponding to 1·01 molecule of sulphur dioxide. When this compound was exposed to the air in an open boat, it was one-half dissociated in 10 minutes. In a similar manner, thiocarbamide (0·1211 g.) absorbed 0·74 molecule of sulphur dioxide in 15 hours, becoming pale yellow; absorption thereafter was very slow. Korczyński and Glebocka (*loc. cit.*) found absorption of 0·5 mol. and described their product as white. In the former case, after the sulphur dioxide had completely gone, the m. p. of the original substance was unchanged. In the latter case, there was a slight depression. A control experiment on ethyl 4(or 5)-glyoxaline-carboxylate showed negligible absorption of sulphur dioxide.

Colour Reactions for Thiolglyoxalines.—The colour reactions of thiolglyoxalines with sulphur dioxide and with auric chloride have been described above. Sato's reaction for thiocarbamide (*loc. cit.*) is applicable to thiolglyoxalines and is best carried out as follows : The substance is dissolved in water, treated with one drop of glacial acetic acid followed by one drop of potassium ferrocyanide solution; a yellowish-green solution is then produced which on warming rapidly becomes intense blue. Tschugaev's reaction (*Ber.*, 1902, **35**, 2483) for thiocarbamide, an intense gentian-blue on warming

with benzophenonechloride, although given by allylthiocarbamide, is not given by 2-thiolglyoxalines. Rheinboldt's reaction for thiol groups (*Ber.*, 1927, **60**, 184)—a wine-red solution—does not apply to 2-thiolglyoxalines or thiocarbamides, which all give yellow solutions. Tanret (*Compt. rend.*, 1909, **149**, 222) described a colour reaction for ergothioneine, which, on warming with chloroform and potassium hydroxide solution, gave a green solution which became blue on acidification. This reaction has been tried on a wide variety of thiolglyoxalines, glyoxalines, and thiocarbamides and is not specific for the thiol group. Thiocarbamides give nothing distinctive and in the glyoxaline group the reaction is probably of value for the diagnosis of constitution. Whilst 2-thiol-4(or 5)-methylglyoxaline gives an eosin-red solution which on careful addition of acid becomes blue, other glyoxalines, when they do give a colour, generally give a yellow or orange-yellow solution which may or may not be discharged by acids. In some cases, the yellow colouring matter is soluble in chloroform. Thus 4(or 5)-methylglyoxaline gives a bright yellow solution, 2-thiol-1 : 4-dimethylglyoxaline gives a yellow solution, whilst its isomeride, 2-thiol-1 : 5-dimethylglyoxaline, gives no colour. Again, 2-thiol-1-phenylglyoxaline gives no colour, whereas 2-thiol-1-phenyl-4-methylglyoxaline gives a bright yellow colour in the aqueous and chloroform layers. The reaction seems to be conditioned by the presence of at least one substituent in the 4- or 5-position, but both may be substituted if carboxyl or carbethoxyl is one, as these probably suffer replacement. Finally, if the hydrogen attached to nitrogen is displaced, only the 1 : 4-disubstituted derivatives produce colour. The reaction is possibly dependent on the formation of the triglyoxaline analogues of the triphenylmethane dyes.

A reaction which appears to be new for thiocarbamides and 2-thiolglyoxalines is the intense blue colour produced when an ammoniacal solution of the substance to be tested is treated with a drop of phosphomolybdic acid. This reagent shows the great ease with which 4(or 5)-carbethoxy-5(or 4)-methyl-2-glyoxaline disulphide is hydrolysed with production of the thiol group by very brief boiling in ammoniacal solution.

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