X. ON THE OXIDATION OF AMINO-ACIDS AND OF RELATED SUBSTANCES WITH CHLORAMINE-T.

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Reference has already been made [1916, 1, 2] to the oxidation of certain amino-acids with hypochlorite and with chloramine-T. The latter term is the abbreviated name for sodio-*p*-toluenesulphochloroamine, a substance which appears to constitute a useful addition to the small class of neutral oxidising agents. These experiments seemed worth extending, partly because interesting products of oxidation were likely to be met with and also as preliminary to a study of the mode of antiseptic action of substances of the chloroamine group. In the case of glycine, alanine, leucine, and α-aminophenylacetic acid it was found that the first product of oxidation, using one molecule of oxidising agent, was an aldehyde, as is usually the case with hypochlorite oxidation, as shown by Langheld. On using two molecules of chloramine-T for the oxidations, nitriles were formed, sometimes in good yield. With histidine, no aldehyde[1] could be isolated, but an excellent yield of cyanomethylglyoxaline was obtained.

The present communication deals especially with the oxidation of the neutral salts of glutamic, aspartic, and methylaspartic acids and asparagine, valine and isoleucine.

Glutamic acid may be considered first since the reaction is less complex than with the other substances.

On acting on a dilute aqueous solution of the mono-sodium salt of glutamic acid (1 mol.) with chloramine-T (1 mol.) an excellent yield of the semi-aldehyde of succinic acid (β-aldehydopropionic acid) is obtained. It is most readily separated and identified by means of its nitrophenylhydrazone, but the substance itself may be isolated and distilled although with considerable

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[1] It would appear that Langheld's observations on the formation of iminazoleacetaldehyde by the action of hypochlorite on histidine require confirmation.

loss, as described by Harries and Alefeld [1909]. The reaction may be expressed as follows:

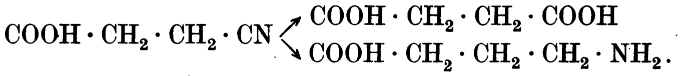
$$\begin{aligned}\mathrm{COOH\cdot(CH_2)_2\cdot CH(NH_2)\cdot COOH} &\rightarrow \mathrm{COOH\cdot(CH_2)_2\cdot CH(NHCl)\cdot COOH}\\ &\rightarrow \mathrm{COOH\cdot(CH_2)_2\cdot CHO + NH_4Cl + CO_2}.\end{aligned}$$

Using two molecules of chloramine-T to one of the glutamic salt, the reaction takes a different course with formation of large quantities of β-cyanopropionic acid, but only negligible traces of succinic acid. The formation of β-cyanopropionic acid is noteworthy since the preparation of this acid has long been unsuccessfully attempted; the methods which would ordinarily be chosen for preparing the substance not having given the desired result.

β-Cyanopropionic acid is a strong monobasic acid, which is readily obtained in crystalline form and with a satisfactory yield. It is likely that it is formed as the result of the decomposition of a dichloroamino-acid, although the latter unstable substance was not isolated.

$$\begin{aligned}\mathrm{COOH\cdot(CH_2)_2\cdot CH(NH_2)\cdot COOH} &\rightarrow \mathrm{COOH\cdot(CH_2)_2\cdot CH(NCl_2)\cdot COOH}\\ &\rightarrow \mathrm{COOH\cdot(CH_2)_2\cdot CN + CO_2 + 2HCl}.\end{aligned}$$

The constitution of the cyano-acid was definitely fixed by its quantitative conversion into succinic acid on treatment with concentrated hydrochloric acid and by its reduction to γ-aminobutyric acid by sodium and alcohol. The latter substance was obtained in very small yield but its formation is of interest since it has been obtained by the bacterial decomposition of glutamic acid.



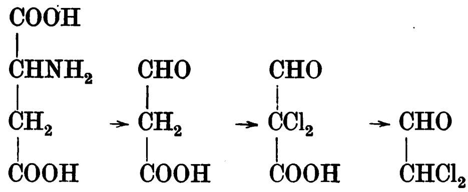
$$\mathrm{COOH\cdot CH_2\cdot CH_2\cdot CN}\begin{cases}\nearrow \mathrm{COOH\cdot CH_2\cdot CH_2\cdot COOH}\\ \searrow \mathrm{COOH\cdot CH_2\cdot CH_2\cdot CH_2\cdot NH_2}.\end{cases}$$

Aspartic acid. The oxidation of aspartic acid, or rather its neutral sodium salt, proved to be a more complicated process. On adding chloramine-T in the proportion of one, two or three molecules to sodium aspartate (1 mol.) in 1–5 % aqueous solution, an immediate reaction took place with precipitation of toluenesulphonamide and liberation of ammonia and carbon dioxide. On heating the filtered solution with phenylhydrazine or semicarbazide or with aromatic orthodiamines, derivatives of glyoxal were obtained in large amounts. The best yields were obtained when between two and three molecular proportions of the chloramine-T were used and with the higher limit the yield of carbon dioxide closely approached two molecules. This carbon dioxide undoubtedly came from the two terminal carboxyl groups, leaving the two adjacent carbon groups to furnish the glyoxal derivatives. The apparent direct oxidation of the (CH_2) group to CHO seemed surprising, and eventually another explanation was found. On oxidising asparagine with chloramine-T, among other products a substance was isolated which proved to be dichloroacetamide. This at once indicated the probability that the hydrogen of the (CH_2) group in aspartic acid, on oxidation with chloramine-T was replaced by chlorine. This proved to be the case and the substance yielding the glyoxal derivatives was identified as dichloroacetaldehyde. Similarly it was found that methylaspartic acid,

$$\mathrm{COOH \cdot C(NH_2)(CH_3) \cdot CH_2 \cdot COOH},$$

on oxidation gave derivatives of methylglyoxal which originated from dichloroacetone.

It appears probable that the half aldehyde of malonic acid is the first product of the oxidation of sodium aspartate with chloramine-T and that the former substance in neutral or alkaline solution undergoes chlorination at the methylene group, giving the half aldehyde of dichloromalonic acid which then loses carbon dioxide to give dichloroacetaldehyde.



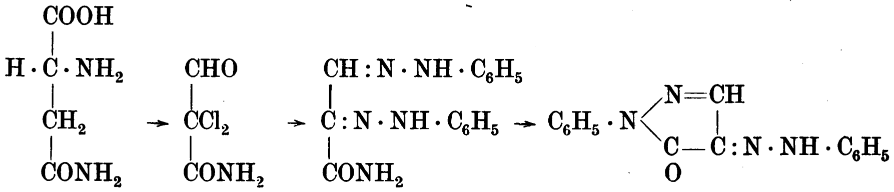
$$\begin{array}{l}\mathrm{COOH}\\ |\\ \mathrm{CHNH_2}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{COOH}\end{array} \rightarrow \begin{array}{l}\mathrm{CHO}\\ |\\ \mathrm{CH_2}\\ |\\ \mathrm{COOH}\end{array} \rightarrow \begin{array}{l}\mathrm{CHO}\\ |\\ \mathrm{CCl_2}\\ |\\ \mathrm{COOH}\end{array} \rightarrow \begin{array}{l}\mathrm{CHO}\\ |\\ \mathrm{CHCl_2}\end{array}$$

The ready chlorination of the half aldehyde of malonic acid is not surprising. Apparently chloramine-T chlorinates free acetaldehyde much less readily than is observed in the present reaction, so that it appears improbable that acetaldehyde is a primary product of oxidation which subsequently undergoes chlorination, although the formation of acetaldehyde from aspartic acid by oxidation with sodium hypochlorite or hydrogen peroxide is well established. Unlike glutamic and many other amino-acids, which yield nitriles on oxidation with two molecules of chloramine-T, no indications were obtained of the presence of cyanoacetic acid among the oxidation products of aspartic acid. On the other hand, the presence of a small quantity of a volatile compound, soluble in ether, giving a strong diazo-reaction, was noted. This substance has not yet been identified but appears to be a glyoxaline derivative. The same compound appears to be formed by the interaction of dichloroacetaldehyde and ammonia, and as both of these latter substances are formed in the oxidation of sodium aspartate by chloramine-T, its formation is in a measure accounted for. The action of ammonia on dichloroacetaldehyde invites further study.

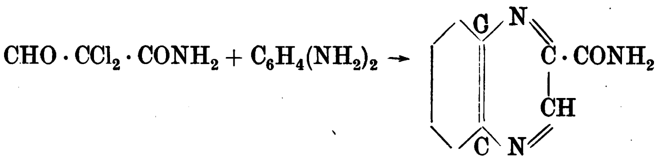
Asparagine. On oxidising aqueous solutions of asparagine with chloramine-T, a reducing substance is formed which, on treatment with phenylhydrazine and other bases, gives derivatives of mesoxalic semi-aldehyde. The substance was not isolated in the pure state but was characterised by a number of derivatives, all of which indicated that its constitution might be represented by some such formula as $CHO \cdot CCl_2 \cdot CO \cdot NH_2$, *i.e.* the semi-aldehyde of dichloromalonamide. In addition, as already mentioned, a considerable quantity of dichloroacetamide was isolated from the oxidation products. Neither formaldehyde nor acetaldehyde was found in appreciable amounts.

On treating solutions containing the semi-aldehyde of dichloromalonamide with phenylhydrazine acetate in the cold, an oily precipitate is first formed due to the formation of the mono-hydrazone, but on warming, chlorine is removed and the whole is converted into a finely crystalline compound, namely the amide of mesoxalic semi-aldehyde *bis*-phenylhydrazone. On heating the latter compound with dilute hydrochloric acid, it is converted into 4-benzeneazo-1-phenyl-5-pyrazolone already obtained by Knorr and by Will.

The changes may be indicated as follows:

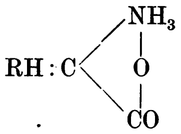


Another reaction of the semi-aldehyde of dichloromalonamide, which may be of practical value, is the fact that it reacts with various orthodiamines to give the amides of quinoxaline-carboxylic acids. These amides as well as many of the corresponding mono-carboxylic acids appear to have been hitherto inaccessible.



Reference must be made here to Langheld's observations on the action of sodium hypochlorite upon asparagine. He states that the product of the reaction is the hitherto unknown half-aldehyde of malonic acid which he did not isolate but characterised as a phenylhydrazone melting at 239–240°. Satisfactory analyses for this compound were recorded by Langheld (see experimental section) but as a matter of fact the compound is the *bis*-phenylhydrazone of mesoxamide semi-aldehyde described above. Direct comparison of the compounds, as well as their mixed melting point and conversion into 4-benzeneazo-1-phenyl-5-pyrazolone prove this conclusively. Langheld's description of the half aldehyde of malonic acid and its hydrazone must therefore be abandoned.

It is a somewhat striking fact that so far as at present observed, chloramine-T does not react at all readily with amino-acid esters. This is the more surprising in view of the pronounced basic properties of the esters. It is possible that chloramine-T reacts with the cyclic betaine form of amino-acids, rather than with the carboxylic form.



In the experimental portion are included experiments demonstrating the formation of isobutyric aldehyde from valine and of methylethylacetaldehyde from isoleucine on oxidation with chloramine-T. These reactions are typical of those previously encountered and present nothing essentially new.

An addendum to the experimental portion gives an account of an interesting substance obtained during the oxidation of carbohydrates by chloramine-T together with a number of its derivatives. The substance proved to be the hitherto unknown benzaldehyde-*p*-sulphonamide. The production of this compound is rather curious. It appears to be produced by the action of chloramine-T upon *p*-toluenesulphonamide, the latter substance being formed by the oxidising action of chloramine-T upon the sugar. The apparent anomaly is thus presented of chloramine-T oxidising its own reaction product. The formation of benzaldehyde-*p*-sulphonamide appears worthy of record since this substance could not be readily synthesised by the usual methods. The other products derived from the glucose are complicated and need further study, although small quantities of saccharic acid have been definitely identified, while little gluconic acid is found, although pentoses are present in noteworthy amount.

EXPERIMENTAL PART.

Oxidation of glutamic acid.

Formation of β-aldehydopropionic acid. This substance is readily formed when neutral sodium glutamate is treated with one molecular proportion of chloramine-T. Glutamic acid (1·47 g.) was dissolved in 100 cc. of hot N/10 sodium hydroxide and the solution cooled to air temperature. Solid chloramine-T (2·9 g.) was next added, which readily dissolved, *p*-toluenesulphonamide being then rapidly precipitated. The reaction did not readily proceed to completion in the cold but on gently warming to 50° a steady evolution of ammonia and carbon dioxide took place and active chlorine, tested for with potassium iodide and acetic acid, rapidly disappeared. The solution was then chilled and the sulphonamide removed by filtration. The filtrate on treatment with nitrophenylhydrazine (1·7 g.) dissolved in hot dilute hydrochloric acid gave 2·1 g. of the nitrophenylhydrazone of β-aldehydopropionic acid compared with a theoretical yield of 2·37 g. The substance was purified by recrystallisation from boiling water and crystallised in platelets which when rapidly heated melted at 185–187°. On analysis the substance was found to contain 17·9 % nitrogen (Dumas) compared with a calculated value of 17·7 for $C_{10}H_{11}O_4N_3$. In the original paper by Harries and Alefeld [1909] this compound was described as melting at 158° and this value later was corrected by Alefeld [1909] who gave 175°.

A second oxidation was carried out with larger quantities of glutamic acid (5·41 g.), but otherwise under similar conditions. After filtering off the sulphonamide, the solution was concentrated under reduced pressure and the filtered concentrated solution acidified with dilute sulphuric acid and extracted thoroughly with ether in a continuous extraction apparatus. On distilling off the ether and distilling the residue under a pressure of 2 mm. a small amount of the oily β-aldehydopropionic acid was obtained as described by Perkin [1899] and by Harries and Alefeld, but by far the greater part of the aldehyde had undergone oxidation during the rather protracted manipulations, so that much succinic acid, m.p. 185°, was obtained in the second fraction. The aldehyde was characterised as nitrophenylhydrazone, m.p. 185–187°, and semicarbazone, m.p. 177–178°.

Formation of β-cyanopropionic acid. Glutamic acid (7·35 g.) was dissolved in 250 cc. 0·2 N sodium hydroxide and chloramine-T (28·4 g. = 2 mols.) added to the cooled solution. The mixture was kept at 35° for a couple of hours, then cooled and filtered from the precipitated sulphonamide. The filtrate was thoroughly extracted with ether in a continuous extraction apparatus to remove remaining traces of sulphonamide, then acidified with sulphuric acid and again extracted to separate the cyano-acid. The second ether extract on evaporation gave 4·75 g. of crude cyanopropionic acid which readily solidified on standing a short time. The substance was almost pure save for a trace of succinic acid which was removed by dissolving the dry residue in a little absolute alcohol, then adding chloroform and a little petroleum ether. The succinic acid was readily precipitated and filtered off and the filtrate on concentration gave a crystalline mass of pure β-cyanopropionic acid which was purified by washing with a little chloroform. The acid is colourless, crystallises in stout prisms and melts at 48–50° without decomposition.

Analysis: 0·1587 g.; 0·2817 g. CO_2; 0·0733 g. H_2O.
0·1058 g.; 13·2 cc. moist N at 20° and 762 mm.

	C	H	N
Found %	48·3	5·1	14·2
Calculated for $C_4H_5O_2N$ %	48·5	5·1	14·1

β-Cyanopropionic acid is a strong acid, freely soluble in most organic solvents, except petroleum ether, and extremely soluble in water, although not deliquescent. 0·111 g. of the acid required 11·1 cc. of N/10 sodium hydroxide to neutralise to phenolphthalein, compared with a calculated value of 11·2 cc. The sodium salt is very soluble in water and aqueous solutions of it are not precipitated by lead acetate, silver nitrate or mercuric chloride.

On evaporation of β-cyanopropionic acid with concentrated hydrochloric acid on the water-bath, it is quantitatively converted into succinic acid, m.p. 185°. The succinic acid was obtained pure on washing the residue with a little cold water to remove ammonium salts and then crystallising from dilute hydrochloric acid.

The reduction of β-cyanopropionic acid was carried out by dissolving 5 g. of the acid in 75 cc. of alcohol and rapidly adding an excess of metallic sodium (7 g.). The mixture was diluted with water, neutralised with dilute sulphuric acid and the alcohol evaporated off on the water-bath. The aqueous solution was then precipitated with phosphotungstic acid in 5 % sulphuric acid. A fine, heavy precipitate was obtained, which after standing was decomposed in the usual way with barium hydroxide. The free γ-aminobutyric acid obtained on evaporation after removing excess of barium hydroxide, was dissolved in hot alcohol and then precipitated with chloroplatinic acid, when the characteristic orange yellow platinum salt, melting at 218–220°, was obtained. (Pt 31·4 %, calculated 31·7 %.) The yield of γ-aminobutyric acid was very poor, amounting to about 5 % of the theoretical amount. It is not unlikely that the conditions of the reduction might be advantageously modified.

Oxidation of aspartic acid.

Aspartic acid was neutralised with 0·1 or 0·2 normal sodium hydroxide and oxidised with chloramine-T, as already directed for glutamic acid, but no essential difference in the nature of the products was noted when either one, two or three molecular proportions of the oxidising agent were used. Quantitative estimations of the carbon dioxide formed in the process showed that 1 gram-molecular proportion of sodium aspartate gave 0·65, 1·43 and 1·8 molecular proportions of carbon dioxide when treated respectively with 1, 2 and 3 gram-molecules of chloramine-T. In each case a volatile aldehyde was present, which proved to be chiefly, if not exclusively, dichloroacetaldehyde.

On treating the filtrates from the toluenesulphonamide with phenylhydrazines either before or after distillation, glyoxal hydrazones derived from dichloroacetaldehyde were readily obtained in fair yield. For example, 1·33 g. aspartic acid was dissolved in 100 cc. 0·1 N sodium hydroxide and treated with 2·84 g. (1 mol.) chloramine-T. The reaction was prompt but active chlorine persisted for some time at room temperature so the mixture was incubated at 35° for half-an-hour. The mixture, which had an odour suggestive of a little isonitrile, was then chilled and the toluenesulphonamide filtered off. On warming the filtrate with excess of phenylhydrazine acetate 0·7 g. of glyoxal *bis*-phenylhydrazone was obtained. Similar experiments with two and three molecular proportions of chloramine-T gave yields of 1·4 and 1·2 g. respectively.

The glyoxal *bis*-phenylhydrazone was crystallised from alcohol and melted at 170–171°. It was free from chlorine and gave the following results on analysis:

0·1025 g.; 0·2650 g. CO_2; 0·0540 g. H_2O.

	C	H	N
Found	70·5	5·85	23·5
Calculated for $C_{14}H_{14}N_4$ %	70·6	5·88	23·5

On similar treatment with *p*-bromophenylhydrazine hydrochloride, glyoxal *bis*-bromophenylhydrazone melting at 240° was obtained, while *p*-nitrophenylhydrazine hydrochloride gave the very characteristic glyoxal *bis*-nitrophenylhydrazone crystallising from pyridine in scarlet needles melting at about 315°.

The dichloroacetaldehyde was also converted into glyoxal *bis*-semicarbazone on digesting the aldehyde solution with excess of semicarbazide hydrochloride and sodium acetate. This reaction has already been observed by Kling [1909]. The product did not melt at 270° and was identical with the substance prepared directly from glyoxal.

Finally, the dichloroacetaldehyde was converted into β-dichlorolactic acid as follows: aspartic acid (13·3 g.) was neutralised with soda and oxidised with chloramine-T (56·8 g. = 2 mols.) as before described. After filtration, the ammonia formed in the reaction was neutralised by addition of acetic acid and the mixture distilled. The distillate was treated with hydrocyanic acid and hydrochloric acid according to the directions of Grimaux and Adam [1880], and after standing over night the nitrile was hydrolysed by boiling. On extraction with ether β-dichlorolactic acid crystallising in platelets and melting at 76–77° was readily obtained.

It is noteworthy that the distillate from the aspartic acid oxidations, especially when free ammonia was not neutralised, gave intense red colour reactions with diazobenzenesulphonic acid in sodium carbonate solution. The same phenomenon was observed when pure dichloroacetaldehyde was distilled with aqueous ammonia, and it seems probable that some glyoxaline synthesis had occurred. The constitution of the compound remains to be determined.

Oxidation of asparagine.

Asparagine was oxidised with chloramine-T in aqueous solution, no addition of alkali being necessary. The reaction commences rapidly at room temperature, with precipitation of toluenesulphonamide. When one or two molecular proportions of chloramine-T are used, all trace of active chlorine disappears on warming the mixture to 30° for about half-an-hour, but with three molecules of chloramine-T the reaction for active chlorine usually persists for a long time even though unchanged asparagine may be present in solution. The products of the reaction are ammonia, carbon dioxide, dichloroacetamide and a non-volatile aldehyde which was not obtained pure, but which is probably the semi-aldehyde of dichloromalonamide,

$$CHO \cdot CCl_2 \cdot CONH_2,$$

from which a variety of derivatives were obtained. The latter will be first described. About 25 % of the theoretical amount of the aldehyde, as judged by its derivatives, was formed when one molecule of chloramine-T was used, about 50 % with two molecules, less with three and almost none with four. The yield of carbon dioxide was about 0·3 molecule for each molecule of oxidising agent.

Mesoxamide semi-aldehyde bis-*phenylhydrazone*,

$$CH : (N \cdot NHC_6H_5)C : (N \cdot NH \cdot C_6H_5) \cdot CONH_2.$$

Asparagine (6·6 g.) was dissolved in hot water (150 cc.), the solution cooled to room temperature and chloramine-T (28·4 g.) added. The reaction went smoothly, and after standing for half-an-hour at 30°, all active chlorine had disappeared. The mixture was cooled and the precipitated sulphonamide removed by filtration and the filtrate mixed with phenylhydrazine (10 g.) dissolved in excess of acetic acid. For a few moments there was no precipitation, then an unattractive-looking oil began to separate in the cold, but on warming on the water-bath, a finely granular reddish precipitate was formed. The hydrazone was filtered off and crystallised from boiling ethyl acetate. It forms light yellow prisms and needles melting sharply at 250–252°. The yield of hydrazone was 4·5 g.

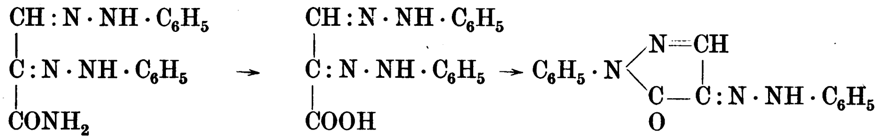
Analysis: 0·1210 g.; 0·2841 g. CO_2; 0·0587 g. H_2O,
0·1500 g.; 33·2 cc. moist N at 19° and 750 mm.

	C	H	N
Found %	64·0	5·38	25·0
Calculated for $C_{15}H_{15}ON_5$ %	64·1	5·32	24·8

The hydrazone is very soluble in warm glacial acetic acid, and moderately soluble in alcohol, ethyl acetate and acetone.

This substance is identical with the compound obtained by Langheld [1909] by the action of sodium hypochlorite upon asparagine to which he erroneously assigned the constitution of a mono-phenylhydrazone of the unknown amide of malonic semi-aldehyde. Langheld gave the melting point of the compound as 239–240° and gave analyses supporting his hypothesis. The calculated values for $C_9H_{11}N_3O_2$ are C 61·04 %, H 6·21 %, N 23·75 %, while Langheld found C 60·81 %, H 5·95 %, N 24·05 %. However, direct comparison of the product obtained according to Langheld's description and the substance above described proved their identity and the agreement of Langheld's values for the carbon and hydrogen with the mono-hydrazone, remains unexplained. The melting point of the product obtained according to Langheld's description was 250–252° and was unchanged on mixing with the *bis*-phenylhydrazone above described and on elementary analysis his compound gave results practically identical with those for the latter substance. On treatment with acids both products gave 4-benzeneazo-1-phenyl-5-pyrazolone.

4-*Benzeneazo*-1-*phenyl*-5-*pyrazolone.* This substance was obtained on dissolving the above described *bis*-phenylhydrazone of the semi-aldehyde of mesoxamide in hot alcohol, adding about 5 % concentrated hydrochloric acid and boiling for two hours under a reflux condenser. On cooling, fine orange reddish needles melting at 150° were obtained, identical with the product obtained by Knorr [1888] and Will [1891] by the action of acetic anhydride or alcoholic hydrochloric acid on mesoxalic semi-aldehyde *bis*-phenylhydrazone. The reaction may be represented as follows:



bis-Phenylhydrazone of semi-aldehyde of mesoxamide

Mesoxalic semi-aldehyde *bis*-phenylhydrazone

4-Benzeneazo-1-phenyl-5-pyrazolone

The constitution of the osazone from the oxidation products of asparagine is satisfactorily established by this reaction.

Mesoxamide semi-aldehyde bis-p-*bromophenylhydrazone.* This substance was obtained in the same way as the *bis*-phenylhydrazone by using an excess of *p*-bromophenylhydrazine hydrochloride at water-bath temperature. The solid product was filtered off, washed with water and a little warm alcohol. It was recrystallised from a mixture of glacial acetic acid and alcohol and formed golden yellow rosettes of needles melting sharply with decomposition at 274–275°. The substance is sparingly soluble in alcohol or ethyl acetate but readily soluble in acetic acid.

Analysis: 0·1782 g.; 24·2 cc. moist N at 15° and 752 mm.
Found ... ... 15·7 % N.
Calculated for $C_{15}H_{13}ON_3Br_2$ 15·9 % N.

Mesoxamide semi-aldehyde bis-p-*nitrophenylhydrazone.* This substance was prepared like the corresponding bromine derivative. 2 g. of asparagine when oxidised with chloramine-T (8·4 g.) gave, when treated with 3 g. nitrophenylhydrazine hydrochloride, 1·4 g. of the nitrophenylhydrazone.. The compound was obtained as a deep scarlet red precipitate which was washed with alcohol and ether. It is very sparingly soluble in alcohol, ether, acetone, amyl alcohol or pyridine, but can be crystallised from boiling nitrobenzene.

It forms deep red prismatic needles melting at about 340°. A trace of the substance treated with sodium hydroxide and alcohol gives an intense blue solution, a reaction which appears to be characteristic of two adjacent nitrophenylhydrazine groups.

Analysis: 0·1500 g.; 30·5 cc. moist N at 21° and 750 mm.

Found	22·7 % N.
Calculated for $C_{15}H_{13}O_5N_7$	22·7 % N.

Mesoxamide semi-aldehyde bis-*semicarbazone.*

$$CH : (N \cdot NH \cdot CONH_2) \cdot C : (N \cdot NH \cdot CONH_2) \cdot CONH_2.$$

A part of the solution obtained from the oxidation of asparagine with two molecules of chloramine-T, as previously described, was concentrated under reduced pressure, then cooled and filtered from a little toluenesulphonamide. The filtrate was gently warmed with excess of semicarbazide hydrochloride, sodium acetate and a few drops of acetic acid. The semicarbazone was slowly precipitated as a heavy white crystalline substance. It is very sparingly soluble in alcohol or water and when recrystallised from either solvent is obtained in the form of fine felted needles melting at 240°.

Analysis: 0·1072 g.; 42·5 cc. moist N at 17° and 750 mm.

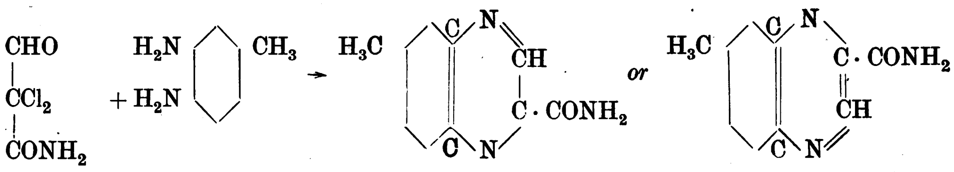
Found	45·1 % N.
Calculated for $C_5H_9O_3N_7$	45·5 % N.

Amide of 7-*methylquinoxaline*-2 *or* 3-*carboxylic acid.* The solution containing the oxidation products from 5 g. of asparagine prepared in the usual way, was treated with excess (3 g.) of 3:4-tolylene diamine. The mixture was acidified decidedly with acetic acid and then warmed on the water-bath for ten minutes. Sodium carbonate was then added to neutralise the acid and the precipitated quinoxaline was allowed to settle out. After a short time the product was filtered off, washed with a little alcohol to remove a small amount of resinous impurity and then crystallised from hot 90 % alcohol. The substance forms glistening plates which appear colourless under the microscope but which are slightly greyish brown in bulk. An alcoholic solution is light yellow in colour. The melting point of the recrystallised substance is 286–287°. The yield was poor, being only about 15 % of the calculated amount.

Analysis: 0·1117 g.; 0·2625 g. CO_2; 0·0483 g. H_2O.

	C	H
Found %	64·1	4·8
Calculated for $C_{10}H_9ON_3$ %	64·2	4·8

The formation of the quinoxaline compound may probably be represented as follows:



Other aromatic orthodiamines react similarly, but the products have not yet been characterised.

Separation of dichloroacetamide from oxidation products of asparagine.

Asparagine (5 g.) was dissolved in water (100 cc.) and oxidised at 20–25° with chloramine-T (25 g.). After two or three hours almost all active chlorine had disappeared and the solution was filtered from toluenesulphonamide and concentrated under reduced pressure to about 30 cc. A small additional separation of sulphonamide was filtered off. The filtrate was next extracted for several hours with ethyl acetate and the solvent was then removed under diminished pressure. Some of the aldehyde compound already discussed is found in the extract but much remains in the aqueous portion. The clear ethyl acetate extract, which often crystallised, was dissolved in a little hot water, treated with a little charcoal, filtered and concentrated slowly. Fine prismatic needles were thus obtained which were purified by dissolving in a little dry ether, filtering from a trace of insoluble matter and allowing the solution to evaporate spontaneously. In this way about a gram of large stout prisms of dichloroacetamide, melting at 96–98°, was obtained. The substance showed all the reactions characteristic of this compound and gave the following results on analysis: C 19·5, H 2·4, N 11·0, calculated for $C_2H_3ONCl_2$, C 18·9, H 2·34, N 10·9.

Oxidation of methylaspartic acid.

Methylaspartic ester was prepared by the action of potassium cyanide and ammonium chloride upon ethyl acetoacetate according to the directions of Zelinsky, Annenkoff and Kulikoff [1911]. The ethyl ester, as already mentioned, did not react at all readily with chloramine-T. The free acid was prepared by hydrolysing 25 g. of the ester with 40 g. barium hydroxide and 250 cc. of water. The mixture was heated two hours on the water-bath, barium salts were then removed exactly by sulphuric acid and the free acid crystallised from the concentrated aqueous solution.

The acid (7·35 g.) was neutralised with 50 cc. normal sodium hydroxide in 450 cc. of water, and chloramine-T (28·4 g.) was then added. In a few seconds separation of sulphonamide began and the solution became moderately warm. Active chlorine rapidly disappeared from the solution and the odour of dichloroacetone became marked. The solution was neutralised with acetic acid and distilled, when oily drops of dichloroacetone collected in the distillate. It was identified by its reactions with phenylhydrazine, Fehling's and alkaline silver solutions. Another portion was warmed with excess of *p*-nitrophenylhydrazine in dilute sulphuric acid, when a 30 % yield of characteristic methylglyoxal-*bis*-*p*-nitrophenylhydrazone was obtained. The substance was dissolved in boiling pyridine and crystallised by adding an equal volume of absolute alcohol and was again crystallised from nitrobenzene and dried *in vacuo* at 150°. It melted at 300–302° and gave the following result on analysis:

0·1345 g.; 29·5 cc. moist N at 20° and 758 mm.

Found ... ... 24·9 % N.

Calculated for $C_{15}H_{14}O_4N_6$ 24·6 % N.

Oxidation of valine.

Valine (1·17 g.) was oxidised in neutral 1 % aqueous solution with chloramine-T (1 mol.). The reaction was rather slow and the mixture required warming at 37° for an hour and a half before active chlorine disappeared. The solution was then neutralised with acetic acid and the volatile isobutyric aldehyde distilled off into nitrophenylhydrazine sulphate solution. The characteristic orange yellow nitrophenylhydrazone (2·3 g.) was crystallised from alcohol and melted at 132–133°. This derivative has already been described [Dakin, 1908]. The substance was analysed with concordant results.

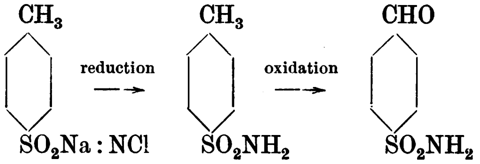
On carrying out the oxidation with two equivalents of chloramine-T, qualitative evidence of the formation of isopropyl cyanide was easily obtained but the yield was too small for satisfactory identification. The formation of this cyanide is analogous to the conversion of leucine into isobutyl cyanide previously referred to in the introduction.

Oxidation of isoleucine.

Synthetic isoleucine (2·62 g.) was oxidised with chloramine-T (5·7 g.) as described in the case of valine. The reaction seemed to go smoothly and ammonia and carbon dioxide were promptly noticeable. The volatile methylethylacetaldehyde was rather difficult to characterise since most of the hydrazine and other derivatives were oily or crystallised with difficulty. Nitrophenylhydrazine was found to give a crystalline derivative, but it was very soluble in most solvents and difficult to crystallise satisfactorily. The aldehyde was therefore oxidised to methylethylacetic acid with moist silver oxide. The silver salt on analysis was found to contain 51·8 % of silver compared with a calculated value of 51·6.

Benzaldehyde-p-*sulphonamide and its derivatives.*

On heating 5 % glucose solutions at water-bath temperature with one, two or three equivalents of chloramine-T, oxidation occurs rather slowly and toluenesulphonamide is precipitated. On treating the filtrate at room temperature with phenylhydrazine acetate, a voluminous yellow precipitate is obtained which at first was thought to be a sugar derivative. The substance on examination proved to be the hydrazone of benzaldehyde-*p*-sulphonamide. The yield of product was considerable, *e.g.* 3 g. glucose when oxidised with 9 g. chloramine-T gave 1·3 g. of pure hydrazone. Apparently the aldehyde is produced from *p*-toluenesulphonamide by oxidation with unchanged chloramine-T; at any rate, its production is noted when the two substances are heated together in aqueous solution.



Subsequently it was found that the aldehyde itself could be separated with ether by prolonged extraction of the aqueous filtrate referred to above. The ether solution on evaporation gave a mixture of the aldehyde with *p*-toluenesulphonamide. The pure aldehyde was most readily obtained by decomposing its "anil" derivative obtained by adding aniline, dissolved in alcohol, to an aqueous extract of the ether residue, or direct to the crude filtrate from the toluenesulphonamide.

p-*Sulphonamidobenzylideneaniline* $(SO_2NH_2) \cdot C_6H_4 \cdot CH : N \cdot C_6H_5$.

This substance, obtained as just described, by adding an alcoholic solution of aniline to an aqueous solution containing benzaldehyde-*p*-sulphonamide, is precipitated as a greyish white precipitate, which on crystallisation from boiling water is obtained as a felted mass of silky crystals melting sharply at 208°. It has the usual properties of anils and is readily decomposed by acids.

Analysis: 0·1168 g.; 0·2563 g. CO_2; 0·0470 g. H_2O;
0·1381 g.; 12·6 cc. moist N at 16° and 766 mm.

	C	H	N
Found %	59·9	4·5	10·7
Calculated for $C_{13}H_{12}O_2N_2S$ %	60·0	4·6	10·8

Benzaldehyde-p-*sulphonamide.* On warming *p*-sulphonamidobenzylideneaniline with ten parts of 10 % sulphuric acid for a few minutes on the water-bath, the aldehyde is readily set free. On repeated extraction with ether, the aldehyde is slowly extracted and is obtained as a crystalline residue on evaporation of the ether. The crude substance is recrystallised from boiling water, filtered from a trace of oily impurity, and is obtained in the form of shining colourless plates and needles melting at 122–124°.

Benzaldehyde-*p*-sulphonamide is readily soluble in warm water, moderately soluble in cold water. It is readily soluble in methyl or ethyl alcohol and acetone but much less soluble in ether. It is sparingly soluble in chloroform, insoluble in petroleum. The aldehyde appears to be quite stable when exposed to the air and undergoes oxidation with some difficulty.

Analysis: 0·1153 g.; 0·1915 g. CO_2; 0·0388 g. H_2O,
0·1000 g.; 6·8 cc. moist N at 17° and 764 mm.

	C	H	N
Found %	45·3	3·7	7·8
Calculated for $C_7H_7O_3NS$ %	45·4	3·8	7·6

Benzaldehyde-p-*sulphonamide hydrazone.* On adding hydrazine hydrochloride to an aqueous solution of benzaldehyde-*p*-sulphonamide a gelatinous precipitate of the hydrazone is obtained which becomes granular on gentle warming and standing. It is very sparingly soluble in boiling water but may be crystallised from 80 % alcohol in which it is moderately soluble. It forms flat shining plates and needles melting at 288–290°.

Analysis: 0·1029 g.; 0·1608 g. CO_2; 0·0405 g. H_2O.

	C	H
Found %	42·6	4·4
Calculated for $C_7H_9O_2N_3S$ %	42·2	4·5

Benzaldehyde-p-*sulphonamide phenylhydrazone.* The formation of this substance has already been described. It is obtained as greenish gold shining plates melting with decomposition at 244–245° when crystallised from hot alcohol in which it is sparingly soluble.

Analysis: 0·1733 g.; 0·3568 g. CO_2; 0·0755 g. H_2O.

	C	H
Found %	56·2	4·8
Calculated for $C_{13}H_{13}O_2N_3S$ %	56·7	4·7

Benzaldehyde-p-*sulphonamide semicarbazone.* This substance was obtained by adding excess of semicarbazide hydrochloride and sodium acetate to an aqueous solution of the aldehyde. The semicarbazone crystallises from water, in which it is sparingly soluble, in the form of characteristic twin prisms and melts at 250–251° with a little sintering a few degrees below. It is not readily soluble in either acid or alkali.

Analysis: 0·1801 g.; 0·2590 g. CO_2; 0·0665 g. H_2O.

	C	H
Found %	39·2	4·1
Calculated for $C_8H_{10}O_3N_4S$ %	39·6	4·1

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