CLXXIII.—*The Preparation of Murexide from Alloxantin and Alloxan.*

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Various samples of murexide prepared for investigation were found to exhibit slight differences in the curves of molecular absorption plotted from their photographed spectra; some of them behaved differently when submitted to the action of heat, and yielded to different solvents traces of other substances. Well-crystallised specimens, apparently pure so far as ultimate organic analysis could determine, showed differences when submitted to spectrographic examination which were proved to be due to impurities.

The composition of the impurities was evidently so very nearly the same as that of murexide that the analytical numbers did not reveal their presence. The details of the various experiments which were carried out are too voluminous for discussion, but it may be stated that a well-crystallised specimen might contain as much as 10 per cent. of foreign matter, and in one instance 50 per cent. was found. For recent work (1904), the compound has been prepared by three different processes, but owing to reasonable doubts as to the constitution of murexide being that usually assigned to it, it became necessary to undertake its investigation. The first question which required solution was the state of hydration of the compound. Two specimens of particularly well-crystallised murexide prepared by Liebig and Wöhler's method were dried *in vacuo* over sulphuric acid in quantities of 1·0 gram and 0·5 gram respectively. They did not lose weight, and were apparently quite unchanged after being heated to 120° in a current of dry air for three hours. These specimens were therefore quite anhydrous. Matignon states that when heated to 100° murexide becomes anhydrous (*Ann. Chim. Phys.*, 1893, [vi], **28**, 289).

New Process of Preparation.—The following process yields pure murexide with greater certainty and facility than that of Liebig and Wöhler. Finely-powdered alloxantin is mixed with about one hundred times its weight of boiling absolute alcohol, and into this a stream of dry ammonia gas is passed, the operation being conducted in a reflux apparatus. A red colour resembling that of murexide is seen after ten minutes, but the operation is continued with advantage for half an hour, and it may be prolonged for three hours or more. The crimson solution is separated from the murexide by filtration, and is concentrated by distilling off half of the ammoniacal solution under reduced pressure.

The murexide on the filter is washed with absolute alcohol, the filtrate and washings being put aside. The solid murexide, which is a purple powder, may be dried in a vacuum or in a water-oven, and the substance obtained in either case is anhydrous. If dissolved in cold water to form a saturated solution, it may be evaporated over sulphuric acid in a vacuum until the murexide is deposited in crystals with the characteristic green metallic lustre. The alcoholic solution filtered from the murexide is generally a perfectly clear liquid, having a colour which varies between crimson and a yellowish-red tint, but its distinguishing character is always a beautiful blue fluorescence visible in daylight. No fluorescence is shown by daylight in solutions of murexide, but a blue fluorescence is seen in quartz cells by the light of an electric spark. This is evidence of a close connection between the two substances. When the alcoholic solution is evaporated to dryness in a vacuum, there remains an orange or scarlet, solid, amorphous substance, which by absorbing moisture from the air becomes changed into a tenacious gummy mass. Eighty per cent. alcohol and even proof spirit may be used for dissolving the ammonia gas, and the murexide obtained is of the same character as that prepared with absolute alcohol, but it is not certain to be of equal purity.

Similarly, coloured products were obtained when solutions of methylamine and ethylamine were substituted for ammonia, but the murexides from these have not yet been examined. The following details show the regularity of the process. Weighed quantities of alloxantin in three experiments were heated for half an hour with from 100 to 150 times their weight of absolute alcohol saturated with ammonia gas; in each case the murexide was filtered off, dried, and weighed; the fluorescent solution and washings were evaporated to dryness and also weighed:

	I.	II.	III.
Alloxantin	0·500 gram	5·00 grams	5·000 grams
Murexide..................	0·329 ,,	3·56 ,,	3·556 ,,
Other product............	0·185 ,,	—	1·720 ,,
Total products	0·514 ,,	—	5·276 ,,

The product of the reaction other than murexide is usually a mixture of a colourless, crystalline substance with blue fluorescence and the amorphous, orange-coloured substance which yields murexide when mixed with aqueous ammonia. Solutions of each isolated substance have been obtained in different reactions, but they are difficult to separate from a mixture on account of the instability of the colourless compound, which on precipitation by ether loses its fluorescent property. It likewise undergoes change when its solution is evaporated to dryness, or even concentrated by evaporation.

The fluorescent substance obtained in the second experiment was not weighed, as it could not be removed from the flask, but we may assume that it was of the same weight as that in the third experiment, since the weight of murexide was the same in each.

The fluorescent solution in different instances gave no further yield of murexide when heated for some time with an additional quantity of alcoholic ammonia.

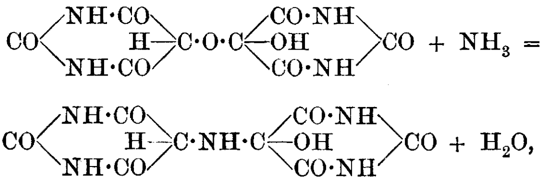
The portion of the fluorescent solution from Experiment II, when evaporated to dryness, yielded a very light powder with a pink tinge, which, when dissolved in alcohol, showed no colour or fluorescence; it gave no colour with caustic potash or ammonia and had evidently altered in constitution. It was thought to be probably uramil, but as it did not combine with alloxan to form murexide this was regarded as evidence to the contrary.

The following explanation of the reaction was based on the observations of von Baeyer, that alloxan and ammonia form mycomelic acid,

$$N_2C_4O_4H_2 + 2NH_3 = N_4C_4O_2H_4 + 2H_2O,$$

in which interaction the mesoxalyl certainly, and the carbonyl possibly, yield oxygen (*Annalen*, 1864, **130**, 174); and that in the production of alloxantin from alloxan and dialuric acid there are three hydroxyls, of which only two are eliminated when the alloxantin is formed. If ammonia acts on it simultaneously, the third hydroxyl is removed as water, and murexide results (von Baeyer, *Annalen*, 1863, **127**, 235). Furthermore, alloxan and uramil combine to form murexide. The initial reaction involved when ammonia acts on alloxantin may be explained by the following equation:

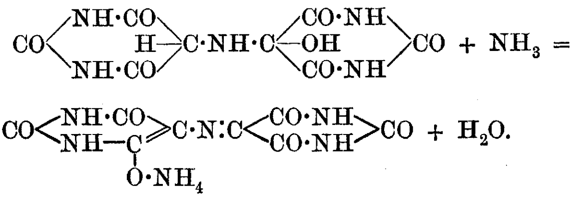
(1)



$$CO\left\langle\begin{array}{l}NH\cdot CO\\ \qquad\quad H\\ NH\cdot CO\end{array}\right\rangle C\cdot O\cdot C\left\langle\begin{array}{l}CO\cdot NH\\ OH\\ CO\cdot NH\end{array}\right\rangle CO + NH_3 =$$

$$CO\left\langle\begin{array}{l}NH\cdot CO\\ \qquad\quad H\\ NH\cdot CO\end{array}\right\rangle C\cdot NH\cdot C\left\langle\begin{array}{l}CO\cdot NH\\ OH\\ CO\cdot NH\end{array}\right\rangle CO + H_2O,$$

but the action of the second molecule of ammonia is open to question. Thus, the simplest way of expressing it is that which results in the formation of murexide:

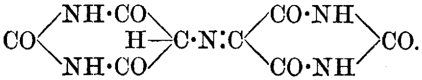
(2)


$$CO\left\langle\begin{matrix}NH\cdot CO\\ NH\cdot CO\end{matrix}\right\rangle H\!\!\rightarrow\!C\cdot NH\cdot C\left\langle\begin{matrix}CO\cdot NH\\ \leftarrow OH\\ CO\cdot NH\end{matrix}\right\rangle CO + NH_3 =$$

$$CO\left\langle\begin{matrix}NH\cdot CO\\ NH-\underset{O\cdot NH_4}{C}\end{matrix}\right\rangle C\cdot N\!:\!C\left\langle\begin{matrix}CO\cdot NH\\ CO\cdot NH\end{matrix}\right\rangle CO + H_2O.$$

When we endeavour to account for the formation of the second and apparently intermediate substance soluble in alcohol with a rich blue fluorescence, it becomes a point of importance to know whether the action of water on it forms either purpuric acid or murexide. In point of fact, it does neither, nor does alcoholic or aqueous ammonia convert it into murexide, and therefore it is not purpuric acid.

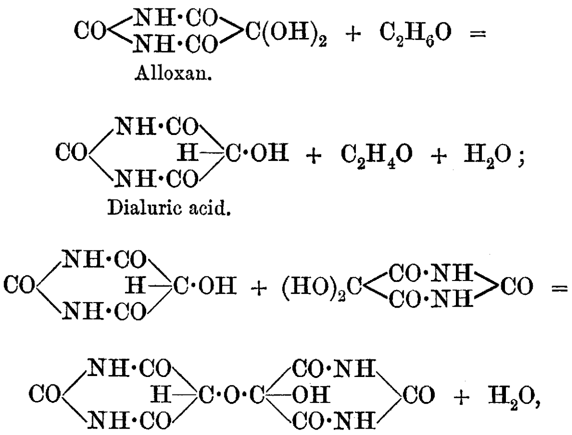
From evidence derived from its absorption spectrum curve, it is believed to be a diureide derivative having probably the formula assigned to the product in equation (1), and by the action of heat on its alcoholic solution a molecule of water is eliminated and a further condensation occurs, yielding the following compound:



$$CO\left\langle\begin{matrix}NH\cdot CO\\ NH\cdot CO\end{matrix}\right\rangle H\!\!\rightarrow\!C\cdot N\!:\!C\left\langle\begin{matrix}CO\cdot NH\\ CO\cdot NH\end{matrix}\right\rangle CO.$$

There is evidence that this is the constitution of the unstable orange-coloured substance which by the action of aqueous ammonia yields murexide. Water alone does not cause this change, nor does the simple addition of alcoholic ammonia. Furthermore, murexide is formed when water is not eliminated in the reaction with ammonia, and this formation could only occur by the operation of a second molecule of ammonia causing the rearrangement within the molecule which results in an ethylenic carbon linking and an ammonium hydroxide salt of the constitution shown in equation (2).

Murexide from Alloxan.—When alloxan is digested with alcoholic ammonia at 78° or thereabout, it becomes converted into a purple powder, insoluble for the most part in alcohol. The alloxan may be first dissolved in boiling absolute alcohol, and may be either the hydrated crystalline form or the recrystallised product dried at 100°; 16 parts readily dissolve in 8 parts of alcohol. The purple solid substance dissolves easily in water to form a solution quite indistinguishable from a solution of murexide. The following is the explanation of the reaction; one molecule of alloxan oxidises alcohol to aldehyde and becomes thereby reduced to dialuric acid, which by the removal of water from another molecule of alloxan combines with it to form alloxantin, on which the alcoholic ammonia reacts in the manner already described:



Alloxan.

Dialuric acid.

Alloxantin.

but it is probable that the oxidation of the alcohol and the condensation of the two ureides to form alloxantin occur simultaneously with an immediate production of murexide. After the reaction, the alcohol separated from the murexide was colourless, but it showed the blue fluorescence before mentioned. The murexide dissolved in cold water yielded crystals on evaporation, with the characteristic green metallic lustre. Owing to the publication of three important papers on the constitution of murexide when my work had reached this stage, the further prosecution of this investigation was suspended.

The researches referred to are those of Piloty and Finckh (*Annalen*, 1904, **333**, 30), R. Möhlau (*Ber.*, 1904, **37**, 2686), and Max Slimmer and Stieglitz (*Amer. Chem. J.*, 1904, **31**, 661). Incidentally it may be remarked that the formula for murexide given in equation (2) is identical with one proposed by Möhlau. The formula for alloxan which I adopted from von Baeyer's work differs from that usually assigned to it, and is also different from that employed by Piloty and the other authors mentioned above. The spectroscopic characters of the intermediate substances appears in the succeeding communication.

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