LXXXIV.—*Triketohydrindene Hydrate. Part III. Its Relation to Alloxan.*

By Siegfried Ruhemann.

The preparation of triketohydrindene hydrate (Trans., 1910, **97**, 1438) from α-hydrindone is a very troublesome task, especially if a large quantity of the substance is required; it therefore became desirable to produce it by a more expedient method. With this object, attempts have been made to prepare the triketone from 1-3-diketohydrindene, since this compound is more readily obtainable

than α-hydrindone. The diketone, indeed, condenses with *p*-nitrosodimethylaniline to yield dimethylaminoanilodiketohydrindene,

$$C_6H_4\begin{<}CO\\CO\end{>}C{:}N\cdot C_6H_4\cdot NMe_2,$$

which, with dilute sulphuric acid, forms triketohydrindene hydrate, but the yield of the anilo-compound is unsatisfactory. Even less convenient for this purpose is β-hydrindone, because this substance can only be procured after considerably greater expenditure of time and trouble than α-hydrindone. After this experience, and owing to the fact that ether has to be used for extracting the triketone from the products of the action of mineral acids on the anilo-compounds, the first method of preparation of the triketone had to be adopted in order to obtain this substance for the continuation of the research.

It was stated previously (Trans., 1910, **97**, 2025) that triketohydrindene hydrate gives a deep blue coloration with amino-acids, and it has since been found that it is a most valuable reagent for proteins and their hydrolytic products, since by means of it mere traces of those substances can be recognised. The isolation of the coloured products which are formed in the reaction presents great difficulties, and, as yet, has not been effected. The fact which has been established that their formation is accompanied by the production of aldehydes points to a similarity in the behaviour between triketohydrindene hydrate and alloxan.

Strecker (*Annalen*, 1862, **123**, 363) showed that, on warming aqueous solutions of a mixture of alloxan and an amino-acid, carbon dioxide is evolved, and, at the same time, aldehydes and murexide are produced. Alloxan, therefore, reacts in these cases as an oxidising agent, and transforms alanine into acetaldehyde, and leucine into valeraldehyde. On using glycine, Strecker was unable to prove the formation of formaldehyde, although, in other respects, the reaction proceeded in the same way as with the former amino-acids; he, therefore, concluded that the formaldehyde underwent oxidation to formic acid or carbon dioxide.

Piloty and Finckh (*Annalen*, 1904, **333**, 68), in the course of their researches on the uric acid group, repeated Strecker's experiment concerning the action of alloxan on glycine, and arrived at the result that, along with uramil, a red-coloured compound was formed, which they regarded as the glycine salt of purpuric acid. This readily decomposed, yielding a brown, amorphous product and a substance which they considered to be uramiloacetic acid,

$$CO\begin{<}NH\cdot CO\\NH\cdot CO\end{>}CH\cdot NH\cdot CH_2\cdot CO_2H.$$

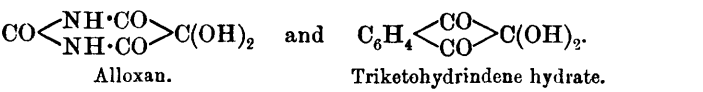
Recently Hurtley and Wootton (this vol., p. 288) studied the interaction of glycine and alloxan again; they state that formaldehyde is evolved, and arrive at views concerning the other products of the reaction which differ from those of Piloty and Finckh.

The behaviour of triketohydrindene hydrate towards amino-acids is similar to that of alloxan; in this case, also, carbon dioxide is evolved and aldehydes are produced. This fact, together with the results of Strecker's experiments and those of Piloty and Finckh, seem to indicate that the blue coloration is preceded by the formation of a substance which resembles alloxantin. Such a compound is readily formed on treatment of the aqueous solutions of triketohydrindene hydrate with hydrogen sulphide, thus:

$$2C_9H_6O_4 + H_2S = C_{18}H_{10}O_6 + 2H_2O + S.$$

This substance may be called *hydrindantin*. It has properties which show a striking resemblance to alloxantin; it is colourless, even less soluble in water than alloxantin, and, like this compound, crystallises with two molecules of water. Of great interest is, first of all, the fact that it yields a blue coloration with amino-acids. This result supports the view expressed above concerning the chemical nature of the reaction which diketohydrindene hydrate gives with amino-acids. Hydrindantin, further, shows the following characteristic behaviour. It dissolves in cold sodium carbonate to form a deep red solution, from which it is precipitated unaltered on the addition of dilute hydrochloric acid. Another salt is produced on using sodium hydroxide (or potassium hydroxide) instead of the carbonate, and this is dark blue. Baryta water, also, gives a blue salt with hydrindantin, as it does with alloxantin. The behaviour of the blue salts of alloxantin and its analogue is alike; both are fairly stable towards heat, but lose their colour under the influence of oxygen. On passing the gas through the blue solution of hydrindantin in potassium hydroxide, the colour disappears in a short time, and the alkaline liquor then contains the salt of *o*-carboxymandelic acid, which, in turn, yields phthalidecarboxylic acid, $C_6H_4\begin{<}{CH(CO_2H)}{CO}\end{>}O$. The acid is also produced by the action of the alkali on triketohydrindene hydrate (Trans., 1910, **97**, 2025), and this fact leads to the view that the formation of the acid from hydrindantin is preceded by the production of the triketone.

The close resemblance in the properties of triketohydrindene hydrate and alloxan finds its expression in the similarity of their formulæ:

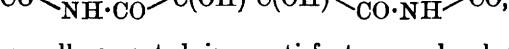


Alloxan. Triketohydrindene hydrate.

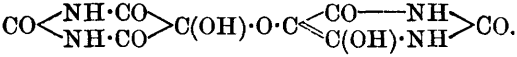
Piloty and Finckh (*loc. cit.*) draw the conclusion that, owing to the property of alloxan to form coloured derivatives with a quinonoid structure, it is itself a quinonoid compound. Hantzsch and Robison (*Ber.*, 1910, **43**, 95) already pointed out that no sufficient evidence exists in support of this view. This follows, also, from the similarity of alloxan to triketohydrindene hydrate; the latter compound is colourless, because it does not contain the quinonoid grouping which is to be found in the diketopyrrolines and their analogues.

Constitution of Alloxantin and Hydrindantin.

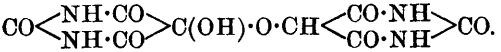
Piloty and Finckh (*loc. cit.*) have shown that the constitution of alloxantin,



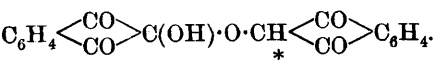
which was generally accepted, is unsatisfactory, and substitute for it the formula:



This they find to be in full agreement with the facts and to express the similarity which they consider to exist between alloxantin and quinhydrone; there is, however, no sufficient reason for such a comparison, nor is it necessary. Piloty and Finckh's formula which, on the whole, accounts for the behaviour of alloxantin, yet requires the following slight alteration:



This formula was suggested before by Slimmer and Stieglitz (*Amer. Chem. J.*, 1904, **31**, 661); it characterises the substance as an alicyclic system which, as such, does not form salts. Indeed, alloxantin yields a violet barium compound, but its production, undoubtedly, is accompanied by a structural change. Accordingly, the constitution of hydrindantin is to be represented thus:



The formation of chromo-salts from hydrindantin and alloxantin may be explained by the change of the diketonic structure into the enolic-

ketonic grouping which is caused by the shifting of the hydrogen marked by an asterisk.

The formula of hydrindantin, however, does not account for the existence of two different sodium salts, namely, a red salt which the compound forms with sodium carbonate, and a blue salt which it yields with sodium hydroxide. Experiments are in progress with the object of arriving at an explanation of this phenomenon.

Experimental.

Formation of Triketohydrindene Hydrate from 1 : 3-Diketohydrindene and β-Hydrindone.

1 : 3-Diketohydrindene, $C_6H_4<\begin{matrix}CO\\CO\end{matrix}>CH_2$, was prepared by following Wislicenus and Kötzle's directions (*Annalen*, 1889, **252**, 72) for the production of ethyl sodio-diketohydrindenecarboxylate and then decomposing this compound in the manner suggested by Kaufmann (*Ber.*, 1897, **30**, 385). The condensation of the diketone with sodio-*p*-nitrodimethylaniline takes place without the use of alkali, simply by mixing the reagents dissolved in hot alcohol. An ebullition occurs, the solution turning deep brown and depositing a dark solid; this dissolves in a large quantity of boiling alcohol to yield a deep violet solution and, on cooling, gradually separates in bluish-green needles, which melt and decompose at 211°. The compound, 2-*Dimethylamino-anilo*-1 : 3-*diketohydrindene*, $C_6H_4<\begin{matrix}CO\\CO\end{matrix}>C{:}N\cdot C_6H_4\cdot NMe_2$, contains water of crystallisation which it loses, slowly, on drying in the air, but rapidly on heating in the water-oven; at the same time, the bluish-green crystals assume a deep bronze colour:

0·2012* gave 0·5405 CO_2 and 0·0930 H_2O. C = 73·26; H = 5·13.
0·2127 ,, 19·2 c.c. N_2 at 20° and 748 mm. N = 10·16.
$C_{17}H_{14}O_2N_2$ requires C = 73·38; H = 5·04; N = 10·07 per cent.

The yield of this substance is unsatisfactory, a large portion of the diketohydrindene being transformed into a black amorphous product which gradually separates from the mother-liquor of the greenish crystals; it readily dissolves in hot alcohol to form a deep red solution and, on cooling, is deposited as a resin. On digesting dimethylamino-anilodiketohydrindene with an excess of dilute sulphuric acid on the water-bath, it decomposes and yields triketohydrindene hydrate. This was isolated by frequent extractions of the dark liquor, which is

* Air-dried.

formed, with ether and, after crystallisation from water, was identified by analysis (Found: C = 60·59; H = 3·66. Calc., C = 60·67; H = 3·37 per cent.) and by its characteristic reactions.

β-Hydrindone may be transformed into triketohydrindene hydrate in the same way as α-hydrindone, and 1 : 3-diketohydrindene. For the preparation of β-hydrindone, Heusler and Schieffer's method (*Ber.*, 1899, **32**, 28) was used, which gives a satisfactory yield, but is very troublesome. The hydrindone readily reacts with *p*-nitrosodimethylaniline on mixing the reagents dissolved in hot alcohol. The solid which separates is only sparingly soluble in alcohol or benzene, but moderately so in chloroform, yielding a deep red solution, from which, on cooling, bronze-coloured needles crystallise out, melting and decomposing at 214°. This substance was not analysed, but there can scarcely be any doubt that it is 1 : 3-bis(*p*-dimethylaminoanilo) β-hydrindone, $C_6H_4<\begin{matrix}C(:N\cdot C_6H_4\cdot NMe_2)\\C(:N\cdot C_6H_4\cdot NMe_2)\end{matrix}>CO$, because it dissolves in dilute sulphuric acid, on warming, to form a dark solution from which triketohydrindene hydrate can be isolated by extraction with ether.

Hydrindantin, $C_6H_4<\begin{matrix}CO\\CO\end{matrix}>C(OH)\cdot O\cdot CH<\begin{matrix}CO\\CO\end{matrix}>C_6H_4$.

This compound is prepared in the same way as alloxantin, namely, by the action of hydrogen sulphide on triketohydrindene hydrate. On passing the gas, at the ordinary temperature, into an aqueous solution of the hydrate, a white precipitate is formed which gradually increases in quantity, and consists of a mixture of hydrindantin and sulphur. After saturation with hydrogen sulphide, the product of the reaction is kept overnight, the solid collected, dried in a vacuum desiccator over sulphuric acid, and then shaken with carbon disulphide. As, by this solvent, the substance cannot be completely freed from sulphur, it must finally be purified by crystallisation. It dissolves in a large quantity of boiling acetone, and separates from the reddish solution in colourless prisms which do not melt, but on heating turn red at 200°, and at 236° decompose completely with evolution of gas.

Hydrindantin, like alloxantin, crystallises with 2 molecules of water, which it loses at 100°.

0·2177* gave 0·4820 CO_2 and 0·0800 H_2O. C = 60·38; H = 4·08.

0·2233, on heating at 100°, lost 0·0228 H_2O. H_2O = 10·21.

$C_{18}H_{10}O_6,2H_2O$ requires C = 60·33; H = 3·91; H_2O = 10·05 per cent.

0·1890† gave 0·4640 CO_2 and 0·0560 H_2O; C = 66·95; H = 3·29.

$C_{18}H_{10}O_6$ requires C = 67·08; H = 3·10 per cent.

* Air-dried. † Dried at 100°.

The compound, when dry, is quite stable, but, in solution it readily undergoes a transformation which is indicated by a colour reaction. On boiling it with alcohol, it gradually dissolves to yield a deep reddish violet solution. This change, undoubtedly, is effected by the ammonia which is present in the air of the laboratory, because the same coloration is produced, at once, on treatment of hydrindantin with ammonia. The substance is only very sparingly soluble in boiling water; it dissolves, however, readily in sodium carbonate to form a dark red solution, from which it is precipitated unaltered on the addition of dilute hydrochloric acid, and loses its colour under the influence of oxygen. If sodium hydroxide is used, instead of the carbonate a deep blue solution is produced which, also, loses its colour on passing oxygen into it for a short time, and then contains the salt of *o*-carboxymandelic acid. If the colourless alkaline liquor is treated with an excess of dilute sulphuric acid and digested on the water-bath, phthalidecarboxylic acid, $C_6H_4<\begin{matrix}CH(CO_2H)\\ \\ CO\end{matrix}>O$ is produced. This was isolated by extraction with ether and identified by the melting point (150—151°) and by analysis. (Found: C = 60·49; H = 3·39. Calc., C = 60·67; H = 3·37 per cent.)

With baryta hydrindantin yields a blue precipitate similar to that given with alloxantin, and, like this, loses its colour under the influence of oxygen.

Triketohydrindene Hydrate as a Reagent for Proteins and their Hydrolytic Products.

It was stated before (*loc. cit.*) that triketohydrindene hydrate reacts with amino-acids, dissolved in water, to yield blue solutions. The reaction has since been applied to a considerable number of substances belonging to this class of compounds with the result that not only α-amino-acids but also the corresponding acids with the amino-group in the β-, γ-, δ-, or ε-positions respond to the test. Most of these compounds give the colour reaction on slightly warming their aqueous solutions with the reagent, with others, such as β-amino-β-phenylpropionic, α-amino*iso*butyric, or α-amino-α-ethylbutyric acids, boiling of the aqueous solutions with the triketone is required. It was pointed out, also, that the reaction depends on the amino-group of the amino-acid being intact; it, therefore, does not occur with phenylglycine or hippuric acid, nor does it take place with proline or hydroxyproline which, instead, give a yellowish-red coloration. Further, it is essential that the carboxyl group of the amino-acid should be intact, for ethyl aminoacetate does not respond to the test. This result is of

importance for the interpretation of the chemical reaction which occurs in the production of the blue colour, because it indicates that diketohydrindene hydrate must first be reduced by the amino-acid (and the latter oxidised to the aldehyde) before the colour can be formed. The reduction product, most probably, is hydrindantin, for this readily yields the blue colour reaction with amino-acids. This fact, together with the results of Piloty and Finckh's experiments (*loc. cit.*) on the interaction of alloxan and glycine, throws a light on the constitution of the coloured products. This question will be dealt with in a subsequent paper.

It is of interest to note the difference in the behaviour of triketohydrindene hydrate towards asparagine and glutamine. Whereas the reagent gives with glutamine, as it does with glutamic acid, the characteristic blue coloration, it yields with asparagine a reddish colour reaction as compared with the blue coloration which it forms with aspartic acid. This fact can only be attributed to the difference in the relative positions of the amino-group to the carbamido-group ($\cdot CO \cdot NH_2$) in asparagine and glutamine. The polypeptides, like the amino-acids, respond to the test, as, indeed, do the proteins.

Physiological Examination of Triketohydrindene Hydrate.

Since, on account of its sensitive colour-reaction with amino-acids and proteins, triketohydrindene hydrate may prove of use in physiological chemistry, it seemed desirable to have some knowledge of its physiological and toxic properties. Preliminary experiments, kindly carried out at my request in a German laboratory, have shown that the substance is markedly poisonous, the fatal dose for frogs being 0·002—0·005 gram. On hypodermic injection, one of the first symptoms is cardiac paralysis. After injection, triketohydrindene hydrate appears to be readily decomposed. In frogs death may ensue without any of the substance passing into the urine, and the characteristic blue coloration was observed, only, at the site of injection (dorsal lymph sac). The substance is an irritant poison; a solution of 1/1024 per cent. still causes a transitory burning sensation when dropped into the eye. Triketohydrindene hydrate is not, however, a general protoplasmic poison, for the growth of yeast is hardly inhibited; on the second day, the culture had a pronounced blue-violet colour which, however, disappeared on the third day.

In conclusion, I wish to express my best thanks to Professors E. Fischer, Gabriel, Sörensen, and to Dr. Colman, for the large number of

amino-acids and polypeptides they placed at my disposal, which made it possible to apply the reagent in the various directions; I am indebted, also, to Professors Kraemer and Pope for supplying the indene which was required for the preparation of β-hydrindone.

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