CCXII.—*Triketohydrindene Hydrate*.

By SIEGFRIED RUHEMANN.

IN a recent paper (this vol., p. 1438) it was shown that the condensation product of α -hydrindone with *p*-nitrosodimethylaniline, on treatment with dilute sulphuric acid, decomposes with the formation of triketohydrindene hydrate. Its formula was represented thus:

according to which the elements of water are united with the 2-ketonic group of triketohydrindene. There cannot be any doubt as to the correctness of this formula, because the union of water with any other ketonic group of the triketone would produce a coloured

compound owing to the proximity of two ketonic groups, whereas triketohydrindene hydrate is colourless, as is also 1:3-diketohydrindene, $C_6H_4 \begin{smallmatrix} \diagup CO \\ \diagdown CO \end{smallmatrix} CH_2$. Further evidence in support of the above formula is the fact that the hydrate, on treatment with phosphorus pentachloride, is transformed into the colourless 2:2-dichloro-1:3-diketohydrindene, $C_6H_4 \begin{smallmatrix} \diagup CO \\ \diagdown CO \end{smallmatrix} CCl_2$. Triketohydrindene also forms additive products with other substances, such as guanidine or benzamidine, which are colourless, and therefore are to be represented by formulæ similar to that of triketohydrindene hydrate. The hydrate further reacts with hydrogen cyanide to yield the unstable cyanohydrin, $C_6H_4 \begin{smallmatrix} \diagup CO \\ \diagdown CO \end{smallmatrix} C(OH) \cdot CN$.

A closer study of the remarkable behaviour of potassium hydroxide towards the hydrate which was described previously (*loc. cit.*, p. 1448) led to the following result. The reaction proceeds in three distinct phases, which are indicated by colour changes. On the addition of the alkali to the crystals of the hydrate, they turn yellow and then dissolve to form a yellow solution; this subsequently becomes blue, even at the ordinary temperature, if the alkali is concentrated. The blue colour, however, is very fugitive, and disappears on dilution with water to yield a colourless solution. On using dilute potassium hydroxide (about 15 per cent.), the blue colour does not appear unless the temperature is raised immediately after the addition of the alkali to the hydrate. The colourless alkaline solution, which represents the last phase of the reaction, contains the potassium salt of *o*-carboxymandelic acid, $CO_2H \cdot C_6H_4 \cdot CH(OH) \cdot CO_2H$, because, on treatment with dilute sulphuric acid, it yields phthalidecarboxylic acid,

The formation of this acid leads to the conclusion that, under the influence of the alkali, the five-carbon ring of triketohydrindene ruptures with the formation of phenylglyoxal-*o*-carboxylic acid, $CO_2H \cdot C_6H_4 \cdot CO \cdot CHO$, which finally undergoes the change to phthalidecarboxylic acid. This behaviour resembles in every respect the change which, by the action of alkalis, phenylglyoxal undergoes to mandelic acid:

The result arrived at in examining the behaviour of triketohydrindene hydrate towards potassium hydroxide supports the view which was expressed before (*loc. cit.*) concerning the product of the action of ammonia on the triketone. The formation of phenylglyoxal-*o*-carboxylic acid is to be regarded as the first change of the triketonic compound

which is produced by the alkali, and this view follows, also, from the fact that the yellow alkaline liquor reduces Fehling's solution. The explanation of the intermediate phase of the reaction which is characterised by the blue colour of the alkaline solution is difficult, because the solution readily loses its colour, and passes into the final phase of the reaction. It is, therefore, only from analogy to the changes which the diketopyrrolines and the compounds with similar structure undergo, on treatment with alkalis, that a view can be expressed concerning the nature of the blue product. The blue solution which, for example, diketodiphenylpyrroline yields with potassium hydroxide was explained (Trans., 1909, 95, 984) by the change into its tautomeric form:

which contains a phenolic group and has an *o*-quinonoid structure. A similar arrangement may be assumed to exist in the potassium compound which is formed in the second phase of the action of the alkali on triketohydrindene hydrate. Accordingly, phenylglyoxal-*o*-carboxylic acid, which is first produced, undergoes ring formation, thus:

in which, also, phenolic groups are associated with an *o*-quinonoid structure. A substance with this constitution may be supposed to yield blue salts with alkalis. These, like the corresponding salts of the diketopyrrolines, are unstable, and, on dilution with water, are transformed into the salts of *o*-carboxymandelic acid:

The further study of triketohydrindene hydrate led to results which appear to be of great interest. It was found that a deep blue colour is produced on warming a mixture of aqueous solutions of this compound and an aliphatic or an aliphatic-aromatic amino-acid which contains the amino-group in the side-chain. As shown below, this reaction has been successfully applied to a number of α -amino-acids, but as yet only two β -amino-acids have been tested, and they were found to differ markedly from the α -amino-acids in their behaviour towards the triketone, because with them the colour reaction takes place less readily, and in the case of β -amino- β -phenylpropionic acid is far less intense than with the corresponding α -amino-acids. No

coloration, however, is produced by the triketone in solutions of aromatic amino-acids which contain the amino-group in the nucleus, nor does it occur with substituted amino-acids, such as phenylglycine or hippuric acid. On the other hand, triketohydrindene hydrate gives a blue reaction with peptone, and this fact in the light of the results indicated above leads to the conclusion that in the peptones, compounds occur which contain the free amino-group of amino-acids.

The same coloration is produced in normal human urine on warming it with an aqueous solution of the reagent. This behaviour agrees with the observations of Abderhalden and Pregl (*Zeitsch. physiol. Chem.*, 1905, **46**, 19; see also Abderhalden and Schittenhelm, *ibid.*, 1906, **47**, 1396), according to which the urine contains a protein-like substance. The authors showed that it does not contain free amino-acids, but that these are formed from it on hydrolysis.

EXPERIMENTAL.

Triketohydrindene hydrate was prepared in the manner described before (*loc. cit.*) by warming slightly the product of the action of *p*-nitrosodimethylaniline and α -hydrindone with dilute sulphuric acid; its extraction from the dark solution which is formed, is more conveniently effected by ethyl acetate than by ether on account of its greater solubility in that solvent.

Additive Product of Triketohydrindene with Guanidine,

This is formed by adding triketohydrindene hydrate (1 gram), dissolved in water, to an aqueous solution of guanidine, obtained from its chloride (0.6 gram) and the calculated quantity of potassium hydroxide. The mixture turns red and soon deposits a colourless solid, which is insoluble in water, alcohol or benzene; it does not melt, but begins to darken at about 190° and finally becomes black:

0.2015 gave 0.4045 CO₂ and 0.0740 H₂O. C = 54.75; H = 4.08.

0.1945 „ 32.6 c.c. N₂ at 20° and 754.5 mm. N = 19.02.

C₁₉H₉O₃N₃ requires C = 54.79; H = 4.11; N = 19.18 per cent.

On heating the compound with water for some time, slight decomposition takes place, and a small quantity dissolves to yield a red solution.

Additive Product of Triketohydrindene with Benзамidine,

This is prepared in a similar way to the former substance, namely, by adding sodium carbonate to the mixture of triketohydrindene hydrate (1.2 grams) and benзамidine hydrochloride (1 gram) dissolved in hot water. A white solid is soon precipitated, which is insoluble in water; it is sparingly soluble in boiling alcohol, but does not separate from the solution without the addition of water, when colourless prisms are formed, which darken at about 200° and decompose at 229—230° with evolution of gas:

0.2011 gave 0.5050 CO₂ and 0.0805 H₂O. C = 68.49; H = 4.44.

0.2051 „ 17.8 c.c. N₂ at 17° and 762 mm. N = 10.10.

C₁₆H₁₂O₃N₂ requires C = 68.58; H = 4.29; N = 10.0 per cent.

This substance is insoluble in sodium carbonate; it is decomposed by hot potassium hydroxide with the formation of benzaldehyde.

Additive Product of Triketohydrindene with Hydrogen Cyanide,

Whilst the additive compounds mentioned above are fairly stable, the *cyanohydrin*, which triketohydrindene hydrate forms with hydrogen cyanide, is readily decomposed. It is obtained by mixing the triketone hydrate (0.5 gram), dissolved in water, with potassium cyanide (0.5 gram), and then adding dilute hydrochloric acid to the deep red solution which is produced. The colour disappears and pale brown needles separate which sinter and darken at about 120°, and at 148° melt with evolution of gas. The compound dissolves in boiling water, but at the same time decomposes. For analysis, the crystals formed in the reaction were washed with cold water and dried in a vacuum desiccator over sulphuric acid:

0.2053 gave 13.8 c.c. N₂ at 18° and 749 mm. N = 7.64.

C₁₀H₅O₃N requires N = 7.49 per cent.

Action of Phosphorus Pentachloride on Triketohydrindene Hydrate.

On mixing the triketone hydrate (1 mol.) with phosphorus pentachloride (2 mols.), no reaction takes place at the ordinary temperature; at 100°, the mixture evolves hydrogen chloride and assumes a red colour, which is probably due to the removal of water and the formation of triketohydrindene. The action proceeds more readily if phosphoryl chloride is used together with phosphorus pentachloride; on warming,

the whole dissolves to yield a yellow solution, which is cooled and then poured on ice, when an oil is precipitated which soon solidifies. The solid is washed with light petroleum, which removes a yellow product, whilst a colourless substance remains undissolved. This is readily soluble in hot dilute alcohol, and, on cooling, crystallises in colourless plates. The yield is poor owing to the formation of the yellow by-product. The compound was identified as 2:2-dichloro-1:3-diketohydrindene, $C_6H_4 \begin{smallmatrix} \text{CO} \\ \diagup \quad \diagdown \\ \text{CO} \end{smallmatrix} CCl_2$, by the melting point, 124—125°, and its chemical behaviour (compare Zincke and Gerland, *Ber.*, 1888, 21, 2390).

Formation of Phthalidecarboxylic Acid from Triketohydrindene Hydrate.

The action of potassium hydroxide on the triketone hydrate was described in a previous paper (this vol., p. 1448) and more fully on p. 2026. It was stated that the final phase of the reaction yields a colourless solution. This, when treated with an excess of dilute sulphuric acid and digested on the water-bath for an hour, contains phthalidecarboxylic acid, $C_6H_4 \begin{smallmatrix} \text{CO} \cdot \text{O} \\ \diagup \quad \diagdown \end{smallmatrix} > CH \cdot CO_2H$. It is isolated from the acid solution by extraction with ether, and, on evaporation of the ether, is left as a white solid. This readily dissolves in hot water, and, on cooling, crystallises in colourless plates melting at 150—151°. The yield is almost theoretical. (Found: C=60·70; H=3·37. Calc., C=60·67; H=3·37 per cent.)

The compound is identical with the acid which Zincke (*Ber.*, 1894, 27, 743), in the course of his researches on the action of bleaching powder on quinones, obtained from monochloro- β -naphthaquinone as well as from *isocoumarincarboxylic acid*.

Action of Triketohydrindenehydrate on Amino-acids.

On mixing a slightly warmed aqueous solution of triketohydrindene hydrate and glycine, an intense blue colour develops immediately, and, after a short time, a dark solid separates. The same behaviour is shown by all the α -amino-acids which I have been able to obtain; several of them I owe to the kindness of Dr. Hopkins, whom I have also to thank for the interest he took in the progress of the work. The reaction has been applied to alanine, valine, leucine, tyrosine, to α -amino- β -phenylpropionic, aspartic, and glutamic acids, to tryptophan and cystine. Owing to the fact that the latter compound is sparingly soluble in water, it must be boiled with a solution of the triketone hydrate; in all the other cases

the reaction takes place almost with the same rapidity as with glycine, and requires mere traces of the reagents. The sensitiveness of the reagent is indicated by the fact that the blue colour is still perceptible on slightly warming glycine with a solution which contains 1 part of triketohydrindene hydrate dissolved in 15,000 parts of water. In the same dilution, the reagent yields with ammonia a yellow coloration, whereas the colour is red, or reddish-violet, on using more concentrated solutions (compare this vol., p. 1447). The shade of the blue coloration that is produced shows but slight variations with the different α -amino-acids. Of the β -amino-acids only two have been tested, namely, β -aminopropionic acid and β -amino- β -phenylpropionic acid. The latter, which was prepared according to Posner's directions (*Ber.*, 1903, 36, 4313) by the action of hydroxylamine on cinnamic acid, differs remarkably from its isomeride, α -amino- β -phenylpropionic acid, in its behaviour towards triketohydrindene hydrate; no colour is produced on mixing the reagents dissolved in hot water. A faint violet coloration, however, develops if the solution is boiled for some time; on the other hand, the corresponding α -amino-acid readily reacts with the triketonic compound to yield a deep blue colour. The difference between α - and β -aminopropionic acids in their behaviour towards the reagent, although not so striking as with the former isomeric acids, is yet apparent, especially as regards the rapidity of the reaction, which in this case, also, is greater with the α -amino-acid. It is, however, necessary to examine a larger number of the β -amino-acids before, by means of the reagent, a general distinction between the two groups of amino-acids can be established. Neither phenyl-aminoacetic acid (phenylglycine) nor hippuric acid yield a coloration with the triketone hydrate, even on boiling the aqueous solutions of the mixtures, and this result indicates that the reaction depends on the amino-group of the amino-acid being intact. Aromatic amino-acids which contain the amino-group in the nucleus, for example, *o*-aminobenzoic acid, also do not respond to the test.

The chemical nature of the colour reaction has not yet been ascertained, but experiments in this direction are already in progress, and will be recorded shortly.

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