Experiments on the formation of 4(or 5)-L-aminoethylglyoxaline from histidine / by Arthur James Ewins and Frank Lee Pyman.

XLIII.—*Experiments on the Formation of 4(or 5)-β-Aminoethylglyoxaline from Histidine.*

By Arthur James Ewins and Frank Lee Pyman.

4(or 5)-β-Aminoethylglyoxaline, the base derived from the naturally occurring amino-acid histidine (α-amino-β-glyoxaline-4(or 5)-propionic acid) by the removal of carbon dioxide from the latter has recently become of considerable interest and importance on account of its occurrence in extracts of ergot (Barger and Dale, Trans., 1910, **97**, 2592) and very great physiological activity (Dale and Laidlaw, *J. Physiol.*, 1910, **41**, 318). It has hitherto been obtained by two methods: (1) by synthesis; (2) by the action of putrefactive organisms on histidine itself.

The base was first synthesised by Windaus and Vogt (*Ber.*, 1907, **40**, 3691) from β-glyoxaline-4(or 5)-propionic acid by Curtius's method. More recently, Ackermann (*Zeitsch. physiol. Chem.*, 1910, **65**, 504) has succeeded in obtaining a relatively large yield of the base by the putrefaction of histidine.

Neither method is very satisfactory, since the first is somewhat complex and expensive, while the yields are by no means good. The second method is wholly uncertain in its results. The object

of our investigation was therefore to endeavour to find a simple method of obtaining the base directly from histidine, which is comparatively easily obtainable. In this we were only partly successful, since we were able to obtain only moderate yields of the base by the action of acids in sealed tubes at a temperature of from 265° to 270°. This last factor (temperature) made it a matter of very great difficulty to obtain anything like large amounts of the base. The results obtained, however, were deemed of sufficient interest to form the subject of the present communication.

Experiments were first carried out to determine whether, as in the case of the formation of *p*-hydroxyphenylethylamine from tyrosine, carbon dioxide could be removed from histidine by direct heating. It was found, however, that under varying conditions only a very small amount (0·3 to 1·0 per cent.) of base could be obtained, and the method was abandoned.

By directly heating the monobenzoyl derivative of histidine and subsequently hydrolysing, a somewhat better yield (10 to 20 per cent.) of the base was obtained.

The effect of various acids under varying conditions of temperature and concentration was then investigated, and it was found that yields of base amounting to about 25 per cent. of the theoretical could be obtained with concentrated hydrochloric acid, moderately dilute sulphuric acid, and fused potassium hydrogen sulphate under suitable conditions. After heating for three hours with acids at temperatures below 240°, no formation of 4(or 5)-β-aminoethylglyoxaline took place. At about 240° very little of the base was obtained, the main product of the reaction being *r*-histidine, which had previously been prepared by Fränkel (*Beitr. Chem. Physiol. Path.*, 1906, **8**, 156) in a similar manner. As the temperature was raised, the yield of base gradually improved, and reached a maximum (about 25 per cent. of the theoretical) at about 265—270°. Further increase of temperature led to diminished yield of 4(or 5)-β-aminoethylglyoxaline. With phosphoric acid (44 per cent.) at 250°, no base was produced, nor did the use of hydrogen bromide in acetic acid solution at somewhat lower temperatures yield any better results.

The progress of the work was very greatly facilitated by the physiological estimation of the yield of base in many of our experiments. This was kindly undertaken for us by Dr. H. H. Dale and Dr. P. P. Laidlaw, to whom we wish to express our indebtedness and thanks.

During the course of the investigation some hitherto undescribed salts of histidine and 4(or 5)-β-aminoethylglyoxaline were prepared, and are now described.

EXPERIMENTAL.

The Action of Concentrated Hydrochloric Acid on Histidine.

One gram of histidine hydrochloride was heated in a sealed tube with 2 c.c. of concentrated hydrochloric acid to 270° for three hours. The solution was concentrated and neutralised. To the boiling solution was added an excess of solid picric acid. On cooling, a crystalline precipitate separated, which was collected and freed from picric acid by extraction with ether. The residue, when recrystallised from water, gave 0·2 gram of 4(or 5)-β-aminoethylglyoxaline dipicrate, melting at 233—235°.

The Action of Dilute Sulphuric Acid.

(*a*) *At* 265—270°.—Two grams of histidine monohydrochloride were heated in a sealed tube to 265—270° for three hours with 4 c.c. of a 20 per cent. aqueous solution of sulphuric acid. The reaction product (a dark brown liquid) was treated with sodium carbonate solution until no further precipitate separated, filtered, and the filtrate neutralised and concentrated to about 15 c.c. An equal volume of cold saturated aqueous solution of picric acid was added, and the amorphous precipitate quickly collected. To the filtrate was added 1·5 grams of picric acid in hot saturated aqueous solution. A little resinous precipitate was removed from the hot solution, and the crystalline precipitate, which separated on cooling, was recrystallised from hot water. There was thus obtained 0·85 gram of a picrate (m. p. 228—229°), which crystallised in bunched, slightly curved, pointed needles. Repeated recrystallisation did not raise the melting point above 233—234°, and analysis showed the salt to be the hitherto undescribed 4(or 5)-β-*aminoethylglyoxaline monopicrate*:

0·0978 gave 0·1396 CO_2 and 0·0282 H_2O. C = 38·8; H = 3·2.

$C_{11}H_{12}O_7N_6$ requires C = 38·8; H = 3·5 per cent.

The monopicrate, on recrystallisation from a large excess of picric acid solution, readily yielded the dipicrate corresponding in all respects with that described by Windaus and Vogt (*loc. cit.*).

4(or 5)-β-*Aminoethylglyoxaline Dihydrobromide.*

The dihydrobromide was prepared by thoroughly shaking the finely powdered dipicrate with ether and a slight excess of dilute hydrobromic acid until all the dipicrate had disappeared. The aqueous solution of the hydrobromide thus obtained was then freed from picric acid by means of ether, digested with a little animal charcoal, filtered, and evaporated to dryness in a vacuum. The

residual brown gum became crystalline on the addition of absolute alcohol, and the salt was then purified by recrystallisation from this solvent.

The salt forms stout, colourless, prismatic needles, which melt to a brown liquid at 284° (corr.), after gradually darkening and sintering from about 265°. It is very readily soluble in water, but sparingly so in boiling absolute alcohol. It is anhydrous:

0·1200 gave 0·0957 CO_2 and 0·0440 H_2O. C = 21·7; H = 4·1.

$C_5H_9N_3,2HBr$ requires C = 22·0; H = 4·1 per cent.

(*b*) *At* 240—250°.—Seventy grams of histidine monohydrochloride were heated in sealed tubes with 140 c.c. of 20 per cent. sulphuric acid for three hours at 240—250° in quantities of not more than 4 grams of histidine in one tube; even under these conditions tubes representing 23 grams exploded. The reaction product from the remaining tubes was worked up exactly as described above. There was thus obtained 26·7 grams of a picrate, which melted at 180—190°. On extracting with absolute alcohol, the greater portion dissolved, and the sparingly soluble residue, after recrystallisation from water, gave 4·3 grams of 4(or 5)-β-aminoethylglyoxaline dipicrate. The alcoholic extract was evaporated, and the residue, on crystallisation from water, gave 16·6 grams of pure *r*-histidine dipicrate

r-*Histidine dipicrate* crystallises from water in thin, yellow plates, which contain two molecules of water. After drying at 100°, it begins to sinter at 182°, and decomposes at 190° (corr.). It is readily soluble in alcohol or hot water, but sparingly so in cold water:

0·1660 * lost 0·0091 at 100°. H_2O = 5·5.

$C_{18}H_{15}O_{16}N_9,2H_2O$ requires H_2O = 5·5 per cent.

0·1468 † gave 0·1906 CO_2 and 0·0356 H_2O. C = 35·4; H = 2·7.

0·1364 † „ 24·0 c.c. N_2 at 23° and 766 mm. N = 20·5.

$C_{18}H_{15}O_{16}N_9$ requires C = 35·2; H = 2·5; N = 20·6 per cent.

This salt readily gave the dihydrochloride, which sinters and melts at 225° (corr.): Fränkel (*loc cit.*) gives 220°.

r-*Histidine sesquihydrochloride*, $(C_6H_9O_2N_3)_2,3HCl,H_2O$, crystallises in clusters of flat, prismatic needles when the dihydrochloride is crystallised from dilute alcohol (for instance, when it is dissolved in about twice its weight of water, and four times its weight of alcohol is added). This salt melts at 168—170° (corr.), and suffers no loss in weight at 100°:

0·1513 gave 0·1840 CO_2 and 0·0734 H_2O. C = 33·2; H = 5·4

0·1270 „ 0·1530 CO_2 „ 0·0644 H_2O. C = 32·9; H = 5·7.

* Air-dried salt. † Dried at 100°.

0·1570 gave 0·1555 AgCl. Cl = 24·5.
0·1259 „ 0·1251 AgCl. Cl = 24·6.

$(C_6H_9O_2N_3)_2,3HCl,H_2O$ requires C = 32·9; H = 5·3; Cl = 24·3 per cent.

The composition of this salt is peculiar; there is no evidence of a similar salt of the natural histidine (compare Abderhalden and Einbeck, *Zeitsch. physiol. Chem.*, 1909, **62**, 322).

r-*Histidine monopicrate* crystallises from water in large, flat plates, which are sparingly soluble in hot water and almost insoluble in alcohol. This salt decomposes at 180—181° (corr.), after sintering from about 175°. It contains one molecule of water of crystallisation:

0·3983 * lost 0·0185 at 110°. H_2O = 4·6

$C_{12}H_{12}O_9N_6,H_2O$ requires H_2O = 4·5 per cent.

0·1825 † gave 0·2515 CO_2 and 0·0531 H_2O. C = 37·6; H = 3·3.

$C_{12}H_{12}O_9N_6$ requires C = 37·5; H = 3·2 per cent.

When this salt is dissolved in a hot aqueous solution of picric acid, the dipicrate (m. p. 190°) separates on cooling.

For purposes of comparison, attempts were made to prepare a mono- and di-picrate of naturally occurring histidine. Histidine appears, however, to form only a dipicrate.

Histidine dipicrate crystallises from water in long, flat, clear, well-formed leaflets, which sinter at about 80° and melt at 86° (corr.). It appears to contain two molecules of water:

0·1591 * gave 0·1927 CO_2 and 0·0452 H_2O. C = 33·0; H = 3·2.

$C_{18}H_{15}O_{16}N_9,2H_2O$ requires C = 33·3; H = 3·0 per cent.

The Action of Potassium Hydrogen Suphate on Histidine.

Twenty-five grams of potassium hydrogen sulphate were fused in a beaker heated by an oil-bath and 1 gram of histidine monohydrochloride was added; considerable frothing occurred, and the mixture had to be stirred frequently. After heating for various lengths of time at different temperatures, the reaction product was dissolved in water, neutralised with potassium hydroxide, digested with animal charcoal, cooled, and filtered from charcoal and potassium sulphate. The filtrate was again concentrated, separated from more potassium sulphate, made up to a known volume, and the yield of 4(or 5)-β-aminoethylglyoxaline determined physiologically. Heating at an oil-bath temperature of 260—270° for one hour gave the best yield of the desired base, but the maximum only reached about 5 per cent. of the theoretical. A modification of this method, in which histidine monohydrochloride was heated with ten times its

* Air-dried salt. † Dried at 110°.

weight of potassium hydrogen sulphate in sealed tubes at 265—270° for three hours, gave yields of 20 to 25 per cent. of the theoretical, as indicated by physiological estimation.

The Formation of 4(or 5)-β-*Aminoethylglyoxaline from Benzoylhistidine.*

Monobenzoylhistidine was prepared by the Schotten-Baumann method, as indicated by Fränkel (*loc. cit.*). *It was, however, found to be quite unnecessary to carry out the precipitation with mercuric chloride described by him. On neutralising the solution after the reaction is complete, the pure benzoyl derivative separates very quickly on simply keeping.

One gram of benzoylhistidine so obtained was heated in a vacuum at 240° until all frothing ceased. The black, tarry mass was dissolved in 2 c.c. of concentrated hydrochloric acid, and hydrolysed at 180°. The contents of the tube were washed out with water, and the separated benzoic acid collected, the filtrate extracted with ether, and the aqueous solution neutralised. An equal volume of a solution of picrolonic acid in water was added, and the bulky, amorphous, yellow precipitate collected. The filtrate was concentrated to small bulk, and a concentrated alcoholic solution of picrolonic acid added. After some time, 0·47 gram of 4(or 5)-β-aminoethylglyoxaline picrolonate was obtained, crystallising in bunched needles, and melting at 262—264° (Windaus and Vogt, *loc. cit.*, give " about 266° ").

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* H. Pauly. Ber., 1910, 43, 2254 has also described a method of preparing pure benzylhistidine in good yield. It melted at 249° and not 230° as Hänkel gives.