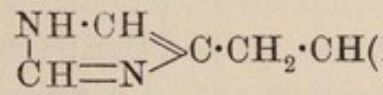
**The synthesis of histidine / by Frank Lee Pyman.**

# CLVII.—*The Synthesis of Histidine.*

By Frank Lee Pyman.

In the following paper an account is given of the synthesis of histidine (*l*-α-amino-β-glyoxaline-4(or 5)-propionic acid), and it may be of interest, in the first place, to recall that this amino-acid is

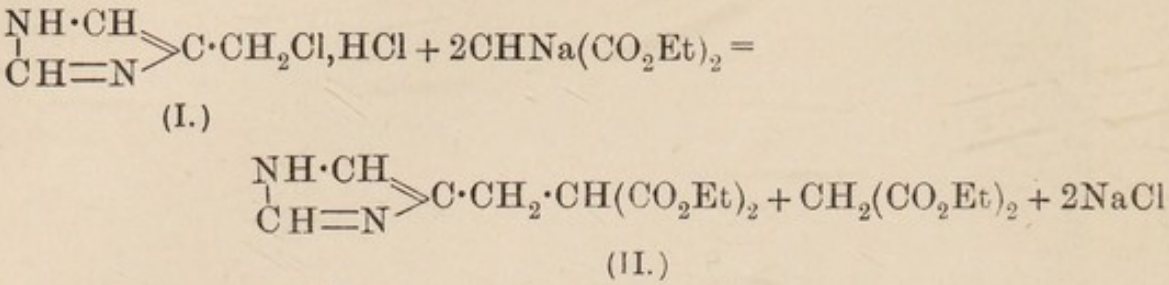


Histidine.

a compound of considerable biochemical importance, since it occurs as a degradation product of nearly all albumins, and notably in large amount by the hydrolysis of hæmoglobin. It was first discovered by Kossel (*Zeitsch. physiol. Chem.*, 1896, **22**, 176), and its constitution was determined mainly by the work of Fränkel (*Monatsh.*, 1903, **24**, 229), Paul (*Zeitsch. physiol. Chem.*, 1904, **42**, 513), and Knoop and Windaus (*Beitr. chem. Physiol. Path.*, 1905, **7**, 144).

In a recent communication (this vol., p. 668) the preparation of certain glyoxaline derivatives was described. It was shown that 4(or 5)-chloromethylglyoxaline (I) is a highly reactive compound, the chlorine atom in the side-chain being readily replaced by hydroxyl under the influence of hot water, and by the cyano-group through the agency of aqueous potassium cyanide at 0°. It therefore seemed probable that this salt could be condensed with compounds of the type of ethyl sodiomalonate, forming the corresponding glyoxalinemethyl, $C_3H_3N_2 \cdot CH_2 \cdot$, derivatives, and this proved to be the case. On adding one molecule of this salt to two molecules of ethyl sodiomalonate, *ethyl* 4(or 5)-*glyoxalinemethylmalonate*

(II) was formed in a yield amounting to 49 per cent. of the theoretical:



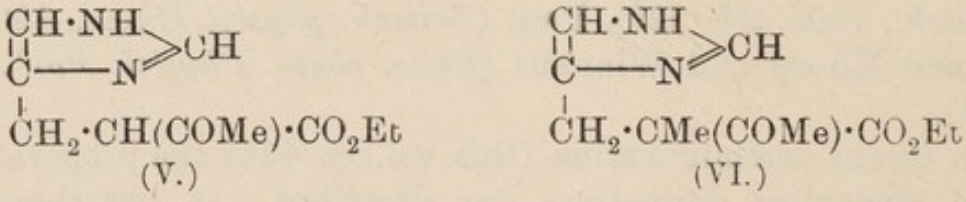
(I.)

(II.)

This ester, on hydrolysis with barium hydroxide, gave the corresponding acid, 4(or 5)-*glyoxalinemethylmalonic acid* (III), together with a certain amount of β-glyoxaline-4(or 5)-propionic acid (IV), produced from the former by the removal of carbon dioxide. 4(or 5)-Glyoxalinemethylmalonic acid is a beautifully crystalline compound, which, on heating, loses carbon dioxide at 180°, and is converted into β-glyoxaline-4(or 5)-propionic acid (IV), a substance which has previously been prepared by Knoop and Windaus (*Beitr. chem. Physiol. Path.*, 1905, **7**, 144), both by the degradation of histidine, and synthetically from glyoxylpropionic acid:

(III.) (IV.)

Similar condensation products were obtained by the action of 4(or 5)-chloromethylglyoxaline hydrochloride on ethyl sodioacetoacetate and ethyl sodiomethylacetoacetate, *ethyl* 4(or 5)-*glyoxalinemethylacetoacetate* (V) and *ethyl* 4(or 5)-*glyoxalinemethylmethylacetoacetate* (VI) being formed:

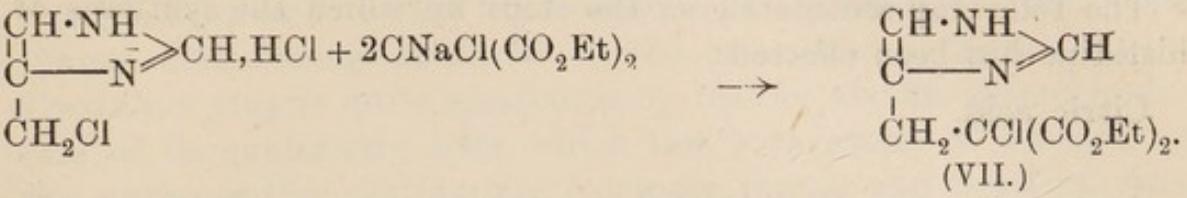


(V.) (VI.)

In view of these results, it seemed of interest to attempt the condensation of this salt with ethyl sodiochloromalonate, since this might lead to a synthesis of histidine, for Conrad (*Annalen*, 1881, **209**, 241) has shown that this ester condenses with benzyl chloride, forming ethyl benzylchloromalonate, although it does not condense with less reactive alkyl chlorides.

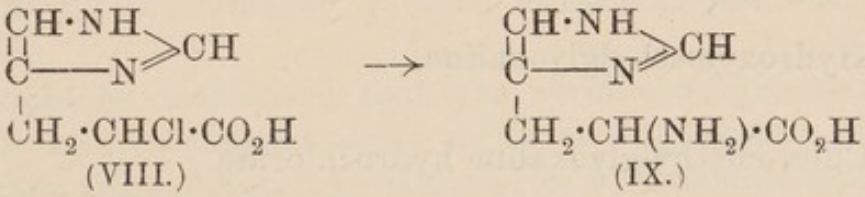
On experiment, it was found that 4(or 5)-chloromethylglyoxaline hydrochloride readily condenses with ethyl sodiochloromalonate,

forming *ethyl* 4(or 5)-*glyoxalinemethylchloromalonate* (VII) in a yield amounting to 60 per cent. of the theoretical:



(VII.)

This ester is readily hydrolysed by boiling 20 per cent. hydrochloric acid, losing two molecules of ethyl alcohol and one of carbon dioxide, and thus becoming almost quantitatively converted into r-*α-chloro-β-glyoxaline*-4(or 5)-*propionic acid* (VIII). This acid, when heated with concentrated aqueous ammonia at 110°, yields r-*α-amino-β-glyoxaline*-4(or 5)-*propionic acid* (IX), that is, *r*-histidine, in a yield amounting to 38 per cent. of the theoretical:



(VIII.) (IX.)

The identity of this synthetic *r*-histidine with that obtained by racemising the naturally occurring lævo-variety (Fränkel, *Beitr. chem. Physiol. Path.*, 1906, **8**, 156; Ewins and Pyman, this vol., p. 339) has been established by analyses of the base and two salts, and by the agreements in the melting points of the base and four salts from either source and the respective mixtures.

The synthesis of histidine itself, that is, the naturally occurring lævo-modification, has been completed by the resolution of the racemic variety. When equimolecular amounts of *r*-histidine and *d*-tartaric acid were crystallised from water, there separated first d-*histidine* d-*hydrogen tartrate* (melting point 234° (corr.); $[\alpha]_D$ +13·3°). This salt is sparingly soluble in water, and is obtained in a yield amounting to about 90 per cent. of the theoretical. The hitherto unknown d-*histidine* was regenerated from it, and found to melt at 287—288° (corr.), and to have $[\alpha]_D$ +39·3°. The mother liquors from the *d*-base-*d*-acid then deposited the easily soluble but magnificently crystalline l-*histidine* d-*hydrogen tartrate* (melting point 172—173° (corr.); $[\alpha]_D$ +17·4°) in a yield amounting to nearly 80 per cent. of the theoretical. The *l*-histidine regenerated from this was found to have $[\alpha]_D$ −36·6°, and was therefore further purified by conversion into the sparingly soluble l-*histidine* l-*hydrogen tartrate* (melting point 234° (corr.); $[\alpha]_D$ −12·1°). After regeneration from this salt, *l*-histidine melted at 287—288° (corr.), and had $[\alpha]_D$ −38·1°.

The specific rotatory power is thus substantially in agreement

with that found for natural histidine, $[\alpha]_D$ −39·7° by Kossel and Kutscher (*Zeitsch. physiol. Chem.*, 1899, **28**, 382).

The following scheme shows the steps by which the synthesis of histidine has been effected:

Citric acid.

↓

Acetonedicarboxylic acid.

↓

Di*iso*nitrosoacetone.

↓

Diaminoacetone hydrochloride.

↓

2-Thiol-4(or 5)-aminomethylglyoxaline.

↓

4(or 5)-Hydroxymethylglyoxaline.

↓

4(or 5)-Chloromethylglyoxaline hydrochloride.

↓

Ethyl 4(or 5)-glyoxalinemethylchloromalonate.

↓

r-α-Chloro-β-glyoxaline-4(or 5)-propionic acid.

↓

*r*-Histidine.

↓

*l*-Histidine.

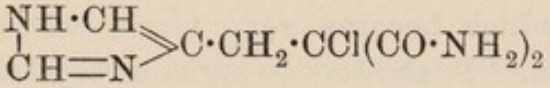
There are a few other points of interest about some of the compounds described. It has already been stated that ethyl 4(or 5)-glyoxalinemethylchloromalonate yields *r*-α-chloro-β-glyoxaline-4(or 5)-propionic acid on hydrolysis with acid, and it was thought that this ester would give the corresponding tartronic acid (X) when boiled with alkali:

(X.)

This, however, was not the case, boiling dilute aqueous sodium hydroxide eliminating one of the nitrogen atoms of the glyoxaline nucleus in the form of ammonia. This remarkable reaction has not yet been further studied, but one other case of a glyoxaline derivative behaving similarly is described in the literature. Thus, Pinner (*Ber.*, 1905, **38**, 2560) found that metapilocarpine—an isomeride of

pilocarpine obtained from the hydrochloride of the latter by prolonged heating at a high temperature—lost half its nitrogen as methylamine when boiled with aqueous potassium hydroxide, nitrogenous acids being produced at the same time. Normally, the glyoxaline ring is quite unaffected by boiling alkalis, except in the case of its quaternary salts, which lose both atoms of nitrogen as the corresponding alkylamines (compare Pinner and Schwarz, *Ber.*, 1902, **35**, 2446).

Ethyl 4(or 5)-glyoxalinemethylchloromalonate gave, with cold alcoholic ammonia, 4(or 5)-*glyoxalinemethylchloromalonamide* (XI), which was isolated in the form of its hydrochloride; strong

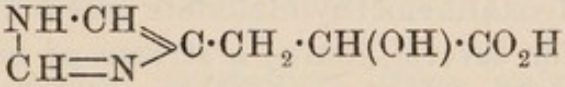


(XI.)

ammonia at 110°, however, gave an uninviting product, which was neglected.

It should be mentioned that the *r*-α-chloro-β-glyoxaline-4(or 5)-propionic acid mentioned above melts at 201° (corr.), that is, 10° higher than the α-chloro-β-glyoxaline-4(or 5)-propionic acid described by Windaus and Vogt (*Beitr. chem. Physiol. Path.*, 1908, **11**, 406). The latter, however, was prepared from *l*-histidine, and is doubtless the corresponding optically active variety.

r-α-*Hydroxy*-β-*glyoxaline*-4(or 5)-*propionic acid* (XII) has also been prepared by the action of hot moist silver oxide on *r*-α-chloro-

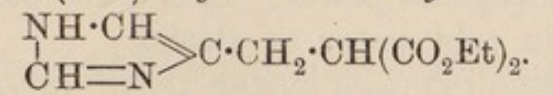


(XII.)

β-glyoxaline-4(or 5)-propionic acid. It melts at 222° (corr.), thus differing from the "oxydeaminohistidine," that is, α-hydroxy-β-glyoxaline-4(or 5)-propionic acid, melting at 204°, prepared by Fränkel (*Monatsh.*, 1903, **24**, 229) by the action of silver nitrite on *l*-histidine. Here again the difference lies, no doubt, in the optical activity of the acid obtained from the natural product. Both acids crystallise with one molecule of water of crystallisation.

## Experimental.

*Ethyl* 4(or 5)-*Glyoxalinemethylmalonate*,



Twenty grams of 4(or 5)-chloromethylglyoxaline hydrochloride were brought into reaction with two molecular proportions of ethyl sodiomalonate, and the product worked up in the same way as that from ethyl sodiochloromalonate (p. 1392); the yield was 21 grams

of *ethyl* 4(or 5)-*glyoxalinemethylmalonate hydrogen oxalate*, that is, 49 per cent. of the theoretical.

*Ethyl* 4(or 5)-*glyoxalinemethylmalonate hydrogen oxalate* crystallises from water in large, hard, clear, nearly rectangular, oblong plates, which melt at 155—158° (corr.). It is anhydrous, sparingly soluble in cold, but readily so in hot, water:

0·1488 gave 0·2564 $CO_2$ and 0·0727 $H_2O$. C = 47·0; H = 5·5.

0·1413 „ 10·6 c.c. $N_2$ at 19° and 778 mm. N = 9·0.

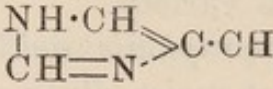
$C_{11}H_{16}O_4N_2,C_2H_2O_4$ requires C = 47·2; H = 5·5; N = 8·5 per cent.

The base was regenerated from the oxalate by means of sodium carbonate and ether; after distillation of the solvent, it remained as a yellow oil, which became very viscid when cold, but readily poured when warm. It showed no signs of crystallisation after long keeping. It is sparingly soluble in water, but readily so in alcohol or ether.

The *hydrochloride* occurs as a deliquescent mass of needles, melting indefinitely at 50—70°, and readily soluble in water, alcohol, acetone, or ethyl acetate.

The *hydriodide* is a crystalline salt of similar properties.

4(or 5)-*Glyoxalinemethylmalonic Acid*,



Ethyl 4(or 5)-glyoxalinemethylmalonate was boiled with an excess of baryta water for several hours; a stream of carbon dioxide was then led through the liquid, and the barium carbonate removed by filtration. The clear filtrate, containing the barium salts of 4(or 5)-glyoxalinemethylmalonic acid and β-glyoxaline-4(or 5)-propionic acid, was then made up to a known volume, and the barium in an aliquot portion determined.

The liquor was then treated with a quantity of oxalic acid exactly sufficient to remove the barium, filtered from barium oxalate, and concentrated to low bulk, when 4(or 5)-glyoxalinemethylmalonic acid crystallised out on cooling; the mother liquor contained β-glyoxaline-4(or 5)-propionic acid.

4(or 5)-*Glyoxalinemethylmalonic acid* crystallises from water in beautiful, clear, hexagonal plates. It is easily soluble in hot water, but sparingly so in cold water or alcohol:

0·1512 gave 0·2530 $CO_2$ and 0·0596 $H_2O$. C = 45·6; H = 4·4.

$C_7H_8O_4N_2$ requires C = 45·6; H = 4·4 per cent.

When heated, this malonic acid melts and effervesces at 180° (corr.), losing carbon dioxide; it resolidifies while still hot, and does not then melt until 207° (corr.), β-glyoxaline-4(or 5)-propionic acid,

$C_3H_3N_2 \cdot CH_2 \cdot CH_2 \cdot CO_2H$, being formed. A quantity of the latter acid was prepared in this manner, and after recrystallisation from water melted at 209—210° (corr.). (Found, C = 51·1; H = 6·0. Calc., C = 51·4; H = 5·8 per cent.) Knoop and Windaus, who have previously prepared this acid in other ways (*Beitr. chem. Physiol. Path.*, 1905, **7**, 144), give m. p. 208—209°.

*Ethyl* 4(or 5)-*Glyoxalinemethylacetoacetate*,
$C_3H_3N_2 \cdot CH_2 \cdot CH(COMe) \cdot CO_2Et$.

This compound was prepared by condensing 4(or 5)-chloromethylglyoxaline hydrochloride (1 mol.) with ethyl sodioacetoacetate (2 mols.).

The *hydrogen oxalate* crystallises from water in rosettes of thin, clear plates, which melt and decompose at 145—146° (corr.). It is anhydrous, fairly readily soluble in cold water, and easily so in hot:

0·1535 gave 0·2704 $CO_2$ and 0·0751 $H_2O$. C = 48·0; H = 5·5.
0·1148 „ 9·3 c.c. $N_2$ at 17° and 754 mm. N = 9·5.
$C_{10}H_{14}O_3N_2, C_2H_2O_4$ requires C = 48·0; H = 5·3; N = 9·3 per cent.

*Ethyl* 4(or 5)-*Glyoxalinemethylmethylacetoacetate*,
$C_3H_3N_2 \cdot CH_2 \cdot CMe(COMe) \cdot CO_2Et$.

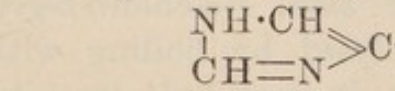
This compound was prepared by condensing 4(or 5)-chloromethylglyoxaline hydrochloride (1 mol.) with ethyl sodiomethylacetoacetate (2 mols.).

The *hydrogen oxalate* crystallises from water in clusters of beautiful, clear, glistening plates, which melt and decompose at 155—156° (corr.). It is anhydrous, fairly readily soluble in cold water, and easily so in hot:

0·1525 gave 0·2874 $CO_2$ and 0·0823 $H_2O$. C = 51·4; H = 6·1.
0·1300 „ 10·2 c.c. $N_2$ at 18° and 762 mm. N = 9·2.
$(C_{11}H_{16}O_3N_2)_4, (C_2H_2O_4)_3$ requires C = 51·4; H = 6·1; N = 9·6 per cent.

On regenerating the base, dissolving it in absolute alcoholic hydrogen chloride, and evaporating nearly to dryness in an evacuated desiccator, a crystalline *hydrochloride* separated in deliquescent needles, which were very easily soluble in water, alcohol, acetone, or ethyl acetate.

*Ethyl* 4(or 5)-*Glyoxalinemethylchloromalonate*,



To 2·2 grams of sodium, dissolved in 200 c.c. of absolute alcohol, 7·8 grams of ethyl chloromalonate were added, followed by a solution

of 30·6 grams of 4(or 5)-chloromethylglyoxaline hydrochloride in 150 c.c. of absolute alcohol, the liquid being kept cold during both additions. The mixture was then boiled for one and a-half hours under a reflux condenser, filtered from sodium chloride, and the solvent removed by distillation. The resulting oil was mixed with dilute hydrochloric acid, and extracted with ether to remove the non-basic esters; the liquor was then rendered alkaline with sodium carbonate, and completely extracted with ether. The residue, after evaporation of the solvent, consisting of a clear, brown oil, was dissolved in a solution of 25 grams of oxalic acid in 550 c.c. of boiling water, and decolorised with animal charcoal. On cooling, 39 grams of pure *ethyl* 4(or 5)-*glyoxalinemethyl chloromalonate hydrogen oxalate* separated, and further small quantities (about 3 grams) were obtained from the mother liquors, the yield thus amounting to 60 per cent. of the theoretical.

*Ethyl* 4(or 5)-*glyoxalinemethylchloromalonate hydrogen oxalate* crystallises from water in shimmering leaflets, which melt and decompose at 176° (corr.). This salt is sparingly soluble in cold water, but readily so in hot. It is anhydrous:

0·1517 gave 0·2442 $CO_2$ and 0·0647 $H_2O$. C = 43·9; H = 4·8.
0·1507 „ 0·2415 $CO_2$ „ 0·0635 $H_2O$. C = 43·7; H = 4·7.
0·2315 „ 0·0991 AgCl. Cl = 10·6.

$(C_{11}H_{15}O_4N_2Cl)_4,(C_2H_2O_4)_3$ requires C = 43·8; H = 4·8; Cl = 10·4 per cent.

The *hydrochloride* crystallises from acetone in beautiful, large, glistening, diamond-shaped plates, which melt at 148—149° (corr.). It is anhydrous, readily soluble in water or alcohol, fairly readily so in hot acetone, and sparingly so in cold acetone:

0·1556 gave 0·2423 $CO_2$ and 0·0739 $H_2O$. C = 42·5; H = 5·3.
$C_{11}H_{15}O_4N_2Cl,HCl$ requires C = 42·4; H = 5·2 per cent.

Aqueous solutions of the hydrochloride give a sparingly soluble precipitate with picric acid or Meyer's solution, and give a deep red coloration with sodium diazobenzene-*p*-sulphonate in alkaline solution. The free base was regenerated by shaking the salts with sodium carbonate and ether; it formed a viscid oil which did not crystallise, and is easily soluble in alcohol, ether, or chloroform, but very sparingly so in water.

Ethyl 4(or 5)-glyoxalinemethylchloromalonate yields, on hydrolysis with 20 per cent. hydrochloric acid, *r*-α-chloro-β-glyoxaline-4(or 5)-propionic acid. When hydrolysed by boiling with dilute aqueous sodium hydroxide, however, it loses half its nitrogen as ammonia. This was determined quantitatively by absorption in dilute sulphuric acid in the usual way.

0·2532 (oxalate) gave $NH_3$ requiring 7·4 c.c. $N/10$-$H_2SO_4$; N = 4·1.

0·5008 (oxalate) gave $NH_3$ requiring 15·2 c.c. $N/10$-$H_2SO_4$; N = 4·3.

$(C_{11}H_{15}O_4N_2Cl)_4,(C_2H_2O_4)_3$ requires total N = 8·2 per cent.

With cold ammonia, it yields 4(or 5)-glyoxalinemethylchloromalonamide, but with strong ammonia at 110° only dark brown, uninviting products are obtained.

*r-α-Chloro-β-glyoxaline*-4(or 5)-*propionic Acid*,

$$\begin{matrix} NH\cdot CH \\ | \\ CH{=}N \end{matrix}\!\!>C\cdot CH_2\cdot CHCl\cdot CO_2H.$$

Ten grams of ethyl 4(or 5)-glyoxalinemethylchloromalonate hydrochloride were boiled with 100 c.c. of 20 per cent. hydrochloric acid for forty-five minutes. The liquor was evaporated to dryness under diminished pressure, moistened with water, and again evaporated to dryness. The resulting colourless varnish was dissolved in 300 c.c. of cold water, digested cold with the silver carbonate from 8 grams of silver nitrate, filtered from silver chloride, and treated with hydrogen sulphide. After the removal of silver sulphide, the filtrate was evaporated to low bulk under diminished pressure, and allowed to crystallise, when 5·1 grams of pure *r*-α-chloro-β-glyoxaline-4(or 5)-propionic acid were obtained; this yield represents 91 per cent. of the theoretical.

r-*α-Chloro-β-glyoxaline*-4(or 5)-*propionic acid* crystallises from water in white, star-like clusters of prismatic needles. It is anhydrous, and melts and decomposes at 201° (corr.), but the melting point varies considerably with the rate of heating, and may be found anywhere between 198° and 204° (corr.). It is sparingly soluble in cold water, alcohol, or acetone, but readily so in hot water:

0·1547 gave 0·2334 $CO_2$ and 0·0571 $H_2O$. C = 41·1; H = 4·1.

0·1604 „ 0·1319 AgCl. Cl = 20·3.

$C_6H_7O_2N_2Cl$ requires C = 41·3; H = 4·1; Cl = 20·3 per cent.

The α-chloro-β-glyoxaline-4(or 5)-propionic acid previously described by Windaus and Vogt (*Beitr. chem. Physiol. Path.*, 1908, **11**, 406) is stated to melt at 191°; it is doubtlessly the optically active variety corresponding with *l*-histidine, from which it was prepared.

*Synthesis of* r-*Histidine.*

$$\begin{matrix} NH\cdot CH \\ | \\ CH{=}N \end{matrix}\!\!>\!C\cdot CH_2\cdot CH(NH_2)\cdot CO_2H.$$

Two and a-half grams of *r*-α-chloro-β-glyoxaline-4(or 5)-propionic acid were dissolved in 50 c.c. of concentrated ammonia (D 0·880), and heated at 110° under pressure for three hours. The liquor was then evaporated to dryness under diminished pressure, and the residue dissolved in a little water and again evaporated. The residue was dissolved in a few c.c. of water, and set aside, when 1·1 grams of r-*histidine monohydrochloride* separated in stout needles, melting at 110—115°; after recrystallisation from water, this salt melted at 117—119° (corr.), after sintering earlier.

A larger quantity of synthetic *r*-histidine was then prepared as follows: Twenty grams of ethyl 4(or 5)-glyoxalinemethylchloromalonate were converted into *r*-α-chloro-β-glyoxaline-4(or 5)-propionic acid hydrochloride by boiling for half an hour with 200 c.c. of 20 per cent. hydrochloric acid, and evaporating the liquor to dryness under diminished pressure.

The resulting colourless varnish was again twice dissolved in water, and evaporated to dryness to remove free hydrochloric acid. It was then dissolved in 240 c.c. of concentrated ammonia (D 0·880), and heated under pressure to 110° for three hours. The liquor was then evaporated to dryness under diminished pressure to remove the excess of ammonia, and the residue dissolved in about 40 c.c. of water. On keeping overnight in an evacuated desiccator over sulphuric acid, the liquor was covered with a crust of ammonium chloride. After the removal of this by filtration, the filtrate began to deposit crystals, and on keeping became semi-solid. The crystals were collected after about half an hour, and, after recrystallisation from water, melted at 117—119° (corr.); they were r-*histidine monohydrochloride*. On concentrating the mother liquors, further crops of this salt and ammonium chloride were obtained; these were separated by fractional crystallisation from water, and a total quantity of 6·3 grams of *r*-histidine monohydrochloride was isolated in a pure state; this yield is 38 per cent. of the theoretical.

*Synthetic* r-*Histidine.*

r-*Histidine monohydrochloride* forms clusters of stout needles (from water), which sinter at 112°, and melt at 117—119° (corr.). It contains two molecules of water of crystallisation, of which only about 1½ molecules are lost at 100°. This salt is easily soluble in water, but sparingly so in alcohol:

0·1556 * lost 0·0196 at 100°. $H_2O$ = 12·6.
0·1530 * gave 0·1781 $CO_2$ and 0·0857 $H_2O$. C = 31·7; H = 6·3.
0·1009 * „ 16·0 c.c. $N_2$ at 16° and 765 mm. N = 18·9.
0·1634 * „ 0·1010 AgCl. Cl = 15·3.

$C_6H_9O_2N_3$,HCl,2$H_2O$ requires C = 31·6; H = 6·2; N = 18·5; Cl = 15·6; and 1½$H_2O$ = 11·9 per cent.

When this salt was dissolved in a little water, and a large excess of absolute alcoholic hydrogen chloride added, the dihydrochloride was precipitated in an amorphous form, but quickly became a crystalline powder on stirring. This salt began to sinter at 230°, and decomposed at 235—236° (corr.):

0·1505 gave 0·1750 $CO_2$ and 0·0638 $H_2O$. C = 31·7; H = 4·8.
0·1088 „ 0·1359 AgCl. Cl = 30·9.

$C_6H_9O_2N_3$,2HCl requires C = 31·6; H = 4·9; Cl = 31·1 per cent.

On dissolving the dihydrochloride in a little water and adding alcohol, the sesquihydrochloride separated on keeping in clusters of prismatic needles, which melted at 168—170° (corr.).

These three hydrochlorides of synthetic *r*-histidine were compared with the corresponding salts of *r*-histidine prepared by racemising *l*-histidine, and found to be identical with them; in each case the corresponding salt and the mixture of the synthetic and racemised salt melted simultaneously.

The melting point of racemised histidine dihydrochloride, given by Fränkel (*loc. cit.*) as 220°, and by Ewins and Pyman (*loc. cit.*) as 225° (corr.), is too low; a re-determination has shown that it should be 235—236° (corr.).

r-*Histidine* was prepared from the synthetic monohydrochloride by digesting it with silver oxide, and filtering to remove silver chloride, removing the excess of silver with hydrogen sulphide, and evaporating to low bulk under diminished pressure. After recrystallisation from water, it formed well defined quadrilateral plates, which decomposed at 283° (corr.) simultaneously with a specimen prepared by racemising *l*-histidine, and a mixture of the two in the same bath. It is anhydrous, and is sparingly soluble in cold water, easily so in hot water, but almost insoluble in absolute alcohol and the other usual organic solvents:

0·1514 gave 0·2551 $CO_2$ and 0·0806 $H_2O$. C = 46·0; H = 6·0.
0·0859 „ 20·0 c.c. $N_2$ at 22° and 763 mm. N = 27·0.

$C_6H_9O_2N_3$ requires C = 46·4; H = 5·9; N = 27·1 per cent.

*r*-Histidine dipicrate was also prepared from the synthetic monohydrochloride, and proved to be identical with the salt described by Ewins and Pyman (*loc. cit.*). It should be mentioned that this

* Air-dried.

salt—from either source—when dried in the air for only a short time, melts at about 103° (corr.), and then, after drying at 100°, sometimes melts between 140° and 150°, although it does not decompose until 190°. When thoroughly air-dried, however, and then dried at 100°, it sinters at about 183°, and melts and decomposes at 190° (corr.).

*Resolution of* r-*Histidine.*

With the object of finding a suitable method for the resolution of synthetic *r*-histidine, some salts of natural histidine with optically active acids were first prepared.

The histidine used for this purpose was prepared from hæmoglobin, and the free base was obtained from its hydrochloride by means of silver carbonate, a method due to Fränkel (*Monatsh.*, 1903, **24**, 229). The base decomposed at 287° (corr.), a temperature considerably higher than that given by Fränkel, namely 253°, and it was therefore analysed. (Found, C = 46·0; H = 6·1. Calc., C = 46·4; H = 5·9 per cent.)

Its specific rotatory power was then determined in a 1-dcm. tube:

0·2, in 10 c.c. of water at 26°, gave $\alpha_D$ −0·74°, whence $[\alpha]_D$ −37·0°.

1·015, in 25 c.c. of water at 28°, gave $\alpha_D$ −1·49°, whence $[\alpha]_D$ −36·7°.

Kossel and Kutscher (*Zeitsch. physiol. Chem.*, 1899, **28**, 382) give $[\alpha]_D$ −39·7°.

It was found that the *d*-camphorsulphonate and neutral *d*-tartrate of this base were very readily soluble in water, and crystallised from this solvent with difficulty.

l-*Histidine* d-*hydrogen tartrate*, however, crystallises from water in beautiful, large, clear, colourless, well defined prisms, often separating in triangular plates with bevelled edges. It is anhydrous, and easily soluble in water. This salt decomposes at 172—173° (corr.):

0·1535 gave 0·2188 $CO_2$ and 0·0718 $H_2O$. C = 38·9; H = 5·2.

$C_6H_9O_2N_3,C_4H_6O_6$ requires C = 39·3; H = 5·0 per cent.

The specific rotatory power of this salt was determined in a 2-dcm. tube; it appears to diminish with increasing concentration:

0·1616, in 15 c.c. of water at 24°, gave $\alpha_D$ +0·37°, whence $[\alpha]_D$ +17·2.

0·5587, in 15 c.c. of water at 24°, gave $\alpha_D$ +1·26°, whence $[\alpha]_D$ +16·9.

0·7474, in 15 c.c. of water at 25°, gave $\alpha_D$ +1·62°, whence $[\alpha]_D$ +16·3.

The base was then regenerated from the pure salt as follows, the

method adopted being substantially that used by Fränkel (*loc. cit.*) for the isolation of histidine from the hydrolytic products of hæmoglobin. The tartrate was dissolved in a large volume of water, and precipitated by mercuric chloride and sodium carbonate; the precipitate was thoroughly washed with water, dissolved in dilute hydrochloric acid, and treated with hydrogen sulphide. After the removal of mercuric sulphide, the liquor was evaporated to dryness under diminished pressure, moistened with water, and again evaporated to dryness to remove free hydrochloric acid. The residue was then dissolved in water, shaken with silver carbonate, filtered from silver chloride, treated with hydrogen sulphide, filtered from silver sulphide, and evaporated to low bulk, when the base crystallised out.

Its specific rotatory power was determined in a 2-dcm. tube:

0·504, in 15 c.c. of water at 22°, gave $\alpha_D$ −2·53°, whence $[\alpha]_D$ −37·7°.

In view of the satisfactory crystalline nature of *l*-histidine *d*-hydrogen tartrate, it was determined to attempt the resolution of synthetic *r*-histidine by fractional crystallisation of the *d*-hydrogen tartrate, and 0·73 gram of synthetic *r*-histidine and 0·7 gram of *d*-tartaric acid were accordingly dissolved in a little water and kept. After a short time there crystallised out 0·6 gram of a sparingly soluble salt, melting at 234° (corr.), which is subsequently shown to be *d*-histidine *d*-hydrogen tartrate, and the mother liquors from this salt, after spontaneous evaporation in a desiccator over sulphuric acid, deposited about 0·2 gram of clear prisms, melting at 172—173° (corr.), which were identical with *l*-histidine *d*-hydrogen tartrate. The resolution of a larger quantity of synthetic histidine was then carried out as follows: 3·5 grams of synthetic *r*-histidine and 3·4 grams of *d*-tartaric acid were dissolved in water, and evaporated to a volume of about 20 c.c., when crystals began to separate from the hot solution. The evaporation was then continued to a volume of about 15 c.c., and the liquor set aside. Clusters of prisms, melting at 234° (corr.), and amounting to 2·9 grams, were then collected, and on concentrating the mother liquor and keeping, a further 0·28 gram of the same salt were obtained.

On recrystallising this salt from water, 3·05 grams of *d*-histidine *d*-hydrogen tartrate were obtained.

d-*Histidine* d-*hydrogen tartrate* crystallises from water in clusters of small prisms, which decompose at 234° (corr.). It dissolves in 25 to 30 parts of cold water, and more readily in hot water. It is anhydrous:

0·1561 gave 0·2237 $CO_2$ and 0·0694 $H_2O$. C = 39·1; H = 5·0.
$C_6H_9O_2N_3,C_4H_6O_6$ requires C = 39·3; H = 5·0 per cent.

Its specific rotatory power was determined in a 2-dcm. tube:

0·9220, in 25 c.c. of water at 28°, gave $\alpha_D$ +0·98°, whence $[\alpha]_D$ +13·3°.

This salt was converted into the free base by the method given above.

d-*Histidine* crystallises from water in beautiful, colourless, monoclinic plates, forming elongated hexagons. It decomposes at 287—288° (corr.), and is anhydrous. It is sparingly soluble in cold water, easily so in hot water, and almost insoluble in absolute alcohol and the other usual organic solvents:

0·1532 gave 0·2608 $CO_2$ and 0·0807 $H_2O$. C = 46·4; H = 5·9.
$C_6H_9O_2N_3$ requires C = 46·4; H = 5·9 per cent.

Its specific rotatory power was determined in a 2-dcm. tube:

0·401, in 15 c.c. of water at 23°, gave $\alpha_D$ +2·10°, whence $[\alpha]_D$ +39·3°.

The mother liquor from the *d*-histidine *d*-hydrogen tartrate was then somewhat concentrated, and inoculated with a trace of the *l*-histidine *d*-hydrogen tartrate obtained in the preliminary experiment mentioned above, when there crystallised 1·65 grams of this salt in clear prisms, decomposing at 172—173° (corr.), and on concentrating the mother liquors, a further 1·05 grams, equally pure. The ultimate mother liquors which continued to deposit crystalline material were neglected.

The melting point of the *l*-base-*d*-acid was unchanged by recrystallising the salt, or mixing it with natural *l*-histidine *d*-hydrogen tartrate. The salt was, however, recrystallised, and its specific rotatory power was determined in a 2-dcm. tube, and found to be in agreement with that of the natural salt at corresponding concentrations:

0·8625, in 25 c.c. of water at 27°, gave $\alpha_D$ +1·20°, whence $[\alpha]_D$ +17·4°.

0·8200, in 15 c.c. of water at 23°, gave $\alpha_D$ +1·76°, whence $[\alpha]_D$ +16·1°.

The recrystallised salt and its mother liquor (=2·7 grams of *l*-base-*d*-acid) were then recombined, and the base regenerated.

The specific rotatory power of the latter was determined in a 2-dcm. tube:

0·4143, in 15 c.c. of water at 26°, gave $\alpha_D$ −2·02°, whence $[\alpha]_D$ −36·6°.

This figure being somewhat low, the whole of the regenerated

base (1·1 grams) was converted into the *l*-hydrogen tartrate, and crystallised from water.

l-*Histidine* l-*hydrogen tartrate* crystallises from water in clusters of prisms, which decompose at 234° (corr.). It is sparingly soluble in cold water. A specimen of this salt prepared from natural histidine had the same melting point and specific rotatory power. The latter was determined in a 2-dcm. tube:

0·6792 (synthetic), in 15 c.c. of water at 22°, gave $\alpha_D$ −1·10°, whence $[\alpha]_D$ −12·1°.

0·6796 (natural), in 15 c.c. of water at 25°, gave $\alpha_D$ −1·10°, whence $[\alpha]_D$ −12·1°.

The synthetic salt was then converted into the free base in the usual way.

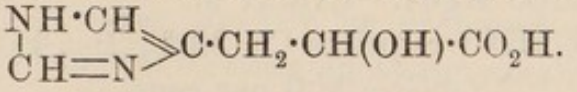
Synthetic *l*-histidine crystallised from water in monoclinic plates, forming elongated hexagons, which decomposed at 287—288° (corr.). Its decomposition point is not depressed by admixture of the base with natural *l*-histidine, but this is of little importance, since it is only depressed about 2° by admixture with *r*-histidine. It is sparingly soluble in cold water, easily so in hot water, and almost insoluble in absolute alcohol and the other usual organic solvents:

0·1402 gave 0·2358 $CO_2$ and 0·0756 $H_2O$. C = 45·9; H = 6·0.
$C_6H_9O_2N_3$ requires C = 46·4; H = 5·9 per cent.

Its specific rotatory power was determined in a 2-dcm. tube:

0·3447, in 15 c.c. of water at 26°, gave $\alpha_D$ −1·75°, whence $[\alpha]_D$ −38·1°.

r-α-*Hydroxy-β-glyoxaline*-4(or 5)-*propionic Acid*,



This acid results from the action of silver hydroxide on a hot aqueous solution of *r*-α-chloro-β-glyoxaline-4(or 5)-propionic acid. After the removal of silver chloride, the solution is treated with hydrogen sulphide, filtered from silver sulphide, and concentrated, when the hydroxy-acid crystallises out.

r-α-*Hydroxy-β-glyoxaline*-4(or 5)-*propionic acid* crystallises from water in prisms, which, after drying at 100°, melt at 222° (corr.). It contains one molecule of water of crystallisation, which is not lost at 100°, and is sparingly soluble in cold water or alcohol, but readily so in hot water:

0·1516 * gave 0·2290 $CO_2$ and 0·0776 $H_2O$. C = 41·2; H = 5·7.
0·1007 * „ 14·0 c.c. $N_2$ at 16° and 755 mm. N = 16·3.
$C_6H_8O_3N_2,H_2O$ requires C = 41·4; H = 5·8; N = 16·1 per cent.

Oxydeaminohistidine, the α-hydroxy-β-glyoxaline-4(or 5)-propionic acid obtained by the action of silver nitrite on *l*-histidine hydrochloride, also crystallises with $1H_2O$. It melts at 204° (Fränkel, *Monatsh.*, 1903, **24**, 229), and is, of course, the optically active variety corresponding with *l*-histidine.

### 4(or 5)-*Glyoxalinemethylchloromalonamide*, $C_3H_3N_2\cdot CH_2\cdot CCl(CO\cdot NH_2)_2$.

One gram of ethyl 4(or 5)-glyoxalinemethylchloromalonate hydrochloride was dissolved in a mixture of 20 c.c. of concentrated ammonia and 10 c.c. of alcohol, and the clear solution kept overnight. It was then evaporated to dryness under diminished pressure, the residue extracted with absolute alcohol, and filtered to remove ammonium chloride, these operations being repeated two or three times. The final alcoholic residue occurred as a varnish, which gave, with absolute alcoholic hydrogen chloride, 0·7 gram of 4(or 5)-*glyoxalinemethylchloromalonamide hydrochloride* as a crystalline precipitate. This salt was dissolved in a little water and mixed with absolute alcoholic hydrogen chloride, and on keeping separated in very pale buff, long, clear spikes, which darkened at 240° and decomposed at 245° (corr.).

It is anhydrous, readily soluble in water, but sparingly so in alcohol:

0·1500 gave 0·1821 $CO_2$ and 0·0567 $H_2O$. C = 33·1; H = 4·2.
0·0805 „ 14·8 c.c. $N_2$ at 18° and 766 mm. N = 21·7.
0·1858 „ 0·2100 AgCl. Cl = 27·9.
$C_7H_9O_2N_4Cl,HCl$ requires C = 33·2; H = 4·0; N = 22·1; Cl = 28·0 per cent.

THE WELLCOME CHEMICAL WORKS,
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* Dried at 100°.

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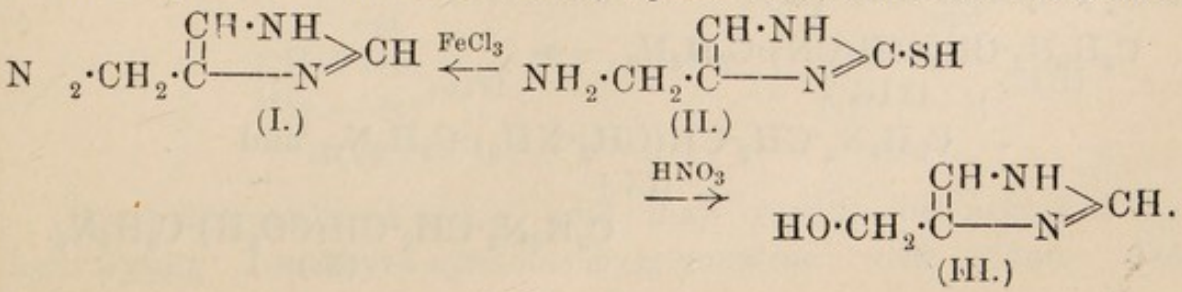
# CCXLV.—*Aminoalkylglyoxalines.*

By Frank Lee Pyman.

In view of the great physiological activity of 4(or 5)-β-aminoethylglyoxaline (compare Dale and Laidlaw, *J. Physiol.*, 1910, **41**, 318), the preparation of several of its homologues has been carried out, and these have been physiologically tested by Dr. P. P. Laidlaw, of the Wellcome Physiological Research Laboratories, to whom the author is indebted for the results given in this paper. In this connexion the recent preparation of other homologues of this base by Ewins (this vol., p. 2052) may be noted.

Barger and Dale (*J. Physiol.*, 1910, **41**, 19), in dealing with the relationship between the chemical constitution of the amines and their physiological action, have shown that the activity varies greatly with the length of the side-chain; in the fatty series the maximum of activity is attained at hexylamine, whilst the most active phenylalkylamine is phenylethylamine, having a fatty side-chain of two carbon atoms. It appeared, therefore, of interest to determine the optimum length of side-chain for physiological effect in the aminoalkylglyoxalines. For comparison with 4(or 5)-β-aminoethylglyoxaline, 4(or 5)-aminomethylglyoxaline and 4(or 5)-γ-aminopropylglyoxaline were required, but since the latter was not readily accessible its methyl homologue, 4(or 5)-γ-aminobutylglyoxaline, was prepared and tested in its place. Compared with 4(or 5)-β-aminoethylglyoxaline, the activity of these bases proved to be negligible.

4(or 5)-Aminomethylglyoxaline (I) has recently been described by Windaus and Opitz (*Ber.*, 1911, **44**, 1721), who prepared it by Curtius' method from glyoxaline-4(or 5)-acetic acid obtained from histidine. It may, however, readily be prepared synthetically by suitably oxidising 2-thiol-4(or 5)-aminomethylglyoxaline (II). It has previously been shown (this vol., p. 669) that the customary method of oxidising thiolglyoxalines to glyoxalines by means of nitric acid leads, in the case of this compound, to 4(or 5)-hydroxymethylglyoxaline (III). If, however, an oxidising agent not producing nitrous acid were employed, the formation of 4(or 5)-aminomethylglyoxaline would be possible. Other oxidising agents were therefore tried, and

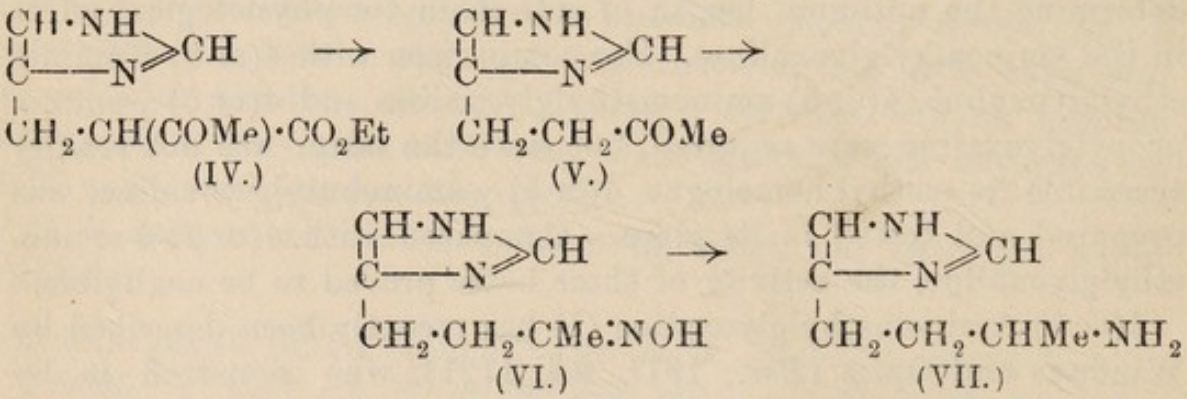


it was found that ferric chloride in calculated quantity oxidised 2-thiol-4(or 5)-aminomethylglyoxaline to 4(or 5)-aminomethylglyoxaline in a yield amounting to more than 50 per cent. of the theoretical:

Potassium permanganate is unsuitable for the oxidation of 2-thiol-4(or 5)-aminomethylglyoxaline to 4(or 5)-aminomethylglyoxaline, for it readily attacks the former substance, causing complete rupture of the glyoxaline ring; cold dilute aqueous solutions of potassium permanganate are immediately decolorised by 2-thiol-4(or 5)-aminomethylglyoxaline, but not by 4(or 5)-aminomethylglyoxaline or other glyoxalines not containing the 2-thiol group. This difference in behaviour is ascribed to the possibility of the thiol base reacting in the tautomeric thiocarbamide form as an unsaturated compound.

4(or 5)-γ-*Aminobutylglyoxaline* was readily prepared as follows:

Ethyl 4(or 5)-glyoxalinemethylacetoacetate (IV) (this vol., p. 1392) was converted into the corresponding ketone, 4(or 5)-γ-*ketobutylglyoxaline* (V), by hydrolysis with hydrochloric acid. This was next transformed into the oxime, 4(or 5)-γ-*oximinobutylglyoxaline* (VI), which on reduction by means of sodium amalgam and acetic acid gave 4(or 5)-γ-*aminobutylglyoxaline* (VII):



A quantity of αβ-bis[4(or 5)-glyoxaline]-propionitrile (VIII) (this vol., p. 677) which is formed as a by-product in the preparation of 4(or 5)-cyanomethylglyoxaline being available, it was thought of interest to reduce it to βγ-*bis*[4(or 5)-*glyoxaline*]-*propylamine* (IX), which may be regarded as an aminoethylglyoxaline containing a glyoxalinemethyl substituent. This reduction was carried out with sodium and alcohol, and the desired base was obtained, together with another compound, which was probably αβ-*bis*[4(or 5)-*glyoxaline*]-*propionic acid* (X):

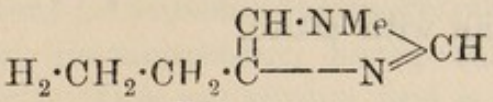
$$C_3H_3N_2\cdot CH_2\cdot CH(CN)\cdot C_3H_3N_2 \longrightarrow$$
(VIII.)

$$C_3H_3N_2\cdot CH_2\cdot CH(CH_2\cdot NH_2)\cdot C_3H_3N_2, \text{ and}$$
(IX.)

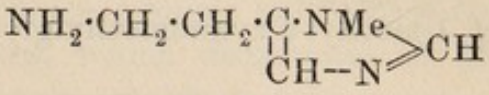
$$C_3H_3N_2\cdot CH_2\cdot CH(CO_2H)\cdot C_3H_3N_2.$$
(X.)

The physiological action of βγ-bis[4(or 5)-glyoxaline]-propylamine is very slight compared with that of 4(or 5)-β-aminoethylglyoxaline.

The two isomeric *N*-methyl derivatives of 4(or 5)-β-aminoethylglyoxaline in which the methyl group substitutes the imino-hydrogen atom of the glyoxaline ring were next prepared. These compounds, 1-methyl-4(and 5)-aminoethylglyoxaline (XI and XII),



(XI.)



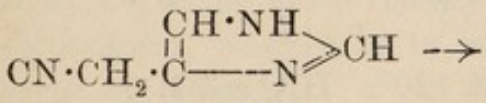
(XII.)

were obtained by reduction of the corresponding methylcyanomethylglyoxalines. Their physiological action is negligible compared with that of 4(or 5)-β-aminoethylglyoxaline.

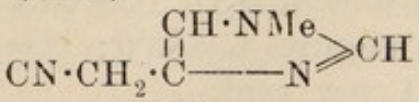
4(or 5)-Cyanomethylglyoxaline (XIII) (this vol., p. 676) yields on methylation with methyl sulphate and alkali a mixture of the 1:4- and 1:5-methylcyanomethylglyoxalines, from which the former can readily be obtained in a pure state, and the latter less readily by fractional crystallisation of the picrates.

1-*Methyl*-4-*cyanomethylglyoxaline* (XIV) gave on reduction with sodium and alcohol 1-*methyl*-4-β-*aminoethylglyoxaline* (XV), together with small quantities of 1:4-dimethylglyoxaline (XVI) and 1-*methylglyoxaline*-4-*acetic acid* (XVII).

1-*Methyl*-5-*cyanomethylglyoxaline* (XVIII) when similarly treated yielded 1-*methyl*-5-β-*aminoethylglyoxaline* (XIX) and 1:5-dimethylglyoxaline (XX). As the constitution of the 1:4- and 1:5-dimethylglyoxalines has already been determined with a high degree of probability (Trans., 1910, **97**, 1814), orientation of the methylated cyanomethylglyoxalines and their other reduction products follows:



(XIII.)



(XIV.)

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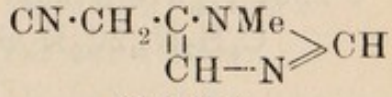
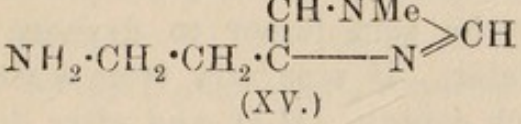
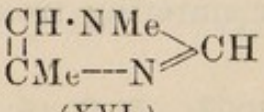


(XVIII.)

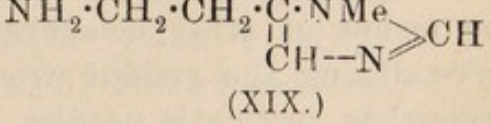


(XV.)

and



(XVI.)



(XIX.)

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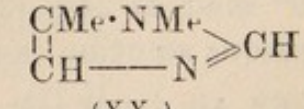
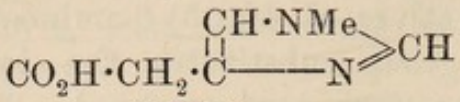


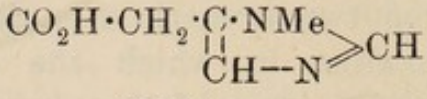
(XX.)

1-Methylglyoxaline-4-acetic acid may readily be prepared by hydrolysing 1-methyl-4-cyanomethylglyoxaline with alkali. Its

ester, *ethyl* 1-*methylglyoxaline*-4-*acetate*, was prepared by the action of alcoholic hydrogen chloride on 1-methyl-4-cyanomethylglyoxaline:



(XVII.)



(XXI.)

1-*Methylglyoxaline*-5-*acetic acid* (XXI) was obtained by hydrolysing 1-methyl-5-cyanomethylglyoxaline with alkali.

## Experimental.

### *Oxidation of* 2-*Thiol*-4(or 5)-*aminomethylglyoxaline to* 4(or 5)-*Aminomethylglyoxaline.*

1·29 Grams of 2-thiol-4(or 5)-aminomethylglyoxaline (this vol., p. 672) were dissolved in 50 c.c. of water, added to a solution of 9·8 grams of ferric chloride in 100 c.c. of water, and the mixture digested for half an hour in the water-bath. Thirty c.c. of 10 per cent. aqueous sodium carbonate were then added, followed by a hot solution of 4·6 grams of picric acid in 100 c.c. of boiling water. The liquor was then boiled with a little animal charcoal and filtered, and on cooling 3·2 grams of pure 4(or 5)-aminomethylglyoxaline dipicrate separated in the first crop; this quantity represents 56 per cent. of the theoretical yield.

4(or 5)-Aminomethylglyoxaline dipicrate crystallises from water in hexagonal or diamond-shaped plates, which melt at 210—211° (corr.). It contains $1H_2O$, which is lost at 120°, but not at 100°. The water of crystallisation in this salt is not mentioned by Windaus and Opitz (*Ber.*, 1911, **44**, 1723), who give the melting point as 209°:

0·1749 * lost *nil* at 100°, lost 0·0054 at 120°. $H_2O = 3·1$.

0·2064 † gave 0·2550 $CO_2$ and 0·0496 $H_2O$. $C = 33·7$; $H = 2·7$.

$C_4H_7N_3,2C_6H_3O_7N_3,H_2O$ requires $C = 33·5$; $H = 2·6$; $H_2O = 3·1$ per cent.

4(or 5)-Aminomethylglyoxaline dihydrochloride was prepared by treating the picrate with hydrochloric acid, removing the picric acid by means of ether, evaporating the acid liquor to dryness, and crystallising the residue from water. It separates from water in colourless, prismatic needles, which contain $\frac{1}{2}H_2O$, and after drying at 100° melt at 244—245° (corr.) after sintering from about 235°. Its aqueous solution is strongly acid:

0·1262, air-dried salt, lost 0·0068 at 100°. $H_2O = 5·4$.

$C_4H_7N_3,2HCl,\frac{1}{2}H_2O$ requires $H_2O = 5·0$ per cent.

* Air-dried. † Dried at 100°.

For anhydrous salt: Found, C = 28·0; H = 5·6. Calc., C = 28·2; H = 5·3 per cent.

Windaus and Opitz (*loc. cit.*), who crystallised this salt from a mixture of methyl alcohol and ether, do not mention any water of crystallisation; they state that on heating, the salt sinters from 236° onwards.

4(or 5)-*Aminomethylglyoxaline hydrogen oxalate* crystallises from water in monoclinic, hexagonal plates, which decompose at 218° (corr.). It is anhydrous, and is sparingly soluble in cold water:

0·2478 gave 0·3158 $CO_2$ and 0·0898 $H_2O$. C = 34·8; H = 4·1.

$C_4H_7N_3,2C_2H_2O_4$ requires C = 34·7; H = 4·0 per cent.

4(or 5)-γ-*Ketobutylglyoxaline* [4(or 5)-*Glyoxaline-ethyl Methyl Ketone*], $CH_3\cdot CO\cdot CH_2\cdot CH_2\cdot C\overset{CH\cdot NH}{\underset{\|}{\phantom{C}}}\!\!>\!CH$.

Fifteen grams of ethyl 4(or 5)-glyoxalinemethylacetoacetate hydrogen oxalate (this vol., p. 1392) were converted into the base, and this boiled under a reflux condenser for three hours with 60 c.c. of hydrochloric acid and 90 c.c. of water. The liquid was then evaporated to dryness under diminished pressure, and the residue dissolved in a little water, mixed with sodium carbonate, and extracted with chloroform. The extract after drying and distillation of the solvent left the ketone as a viscid, brown oil, which solidified on keeping, forming large, buff crystals, melting at 76—78°, and amounting to 4·4 grams; this yield is 64 per cent. of the theoretical.

4(or 5)-γ-*Ketobutylglyoxaline* crystallises from anhydrous ethyl acetate in stout, colourless, prismatic needles, which melt at 80—81° (corr.). It is very readily soluble in water, alcohol, acetone, or chloroform, readily so in ethyl acetate, and very sparingly so in ether or benzene:

0·1278 gave 0·2854 $CO_2$ and 0·0882 $H_2O$. C = 60·9; H = 7·7.

$C_7H_{10}ON_2$ requires C = 60·8; H = 7·3 per cent.

The *picrate* crystallises from water or alcohol in fine, yellow needles, which melt at 192—193° (corr.). It is very sparingly soluble in cold water or alcohol, but fairly readily so in hot water:

0·1088 gave 0·1702 $CO_2$ and 0·0370 $H_2O$. C = 42·6; H = 3·8.

$C_7H_{10}ON_2,C_6H_3O_7N_3$ requires C = 42·5; H = 3·6 per cent.

4(or 5)-γ-*Oximinobutylglyoxaline*, $HO\cdot N{:}CMe\cdot CH_2\cdot CH_2\cdot C\overset{CH\cdot NH}{\underset{\|}{\phantom{C}}}\!\!>\!CH$.

This oxime is readily prepared in good yield by heating on the water-bath an aqueous solution of the ketone with the calculated

amounts of hydroxylamine hydrochloride and sodium carbonate; on concentrating and cooling the solution, the oxime crystallises out, and is purified by recrystallisation from water.

4(or 5)-γ-*Oximinobutylglyoxaline* crystallises from water in clear, colourless plates, which melt at 152—153° (corr.). It is sparingly soluble in cold water or acetone, fairly readily so in alcohol or hot water, but very sparingly so in chloroform:

0·2134 gave 0·4284 $CO_2$ and 0·1407 $H_2O$. C=54·7; H=7·4.

$C_7H_{11}ON_3$ requires C=54·9; H=7·3 per cent.

The *picrate* crystallises from water in hard, spherical grains, which melt at 166—168° (corr.). This salt is sparingly soluble in cold, but readily so in hot, water.

4(or 5)-γ-*Aminobutylglyoxaline*,

$$CH_3\cdot CH(NH_2)\cdot CH_2\cdot CH_2\cdot C(=CH\cdot NH)-N>CH.$$

2·3 Grams of 4(or 5)-γ-oximinobutylglyoxaline were dissolved in 15 c.c. of alcohol and 5 c.c. of glacial acetic acid. Fifty grams of 3 per cent. sodium amalgam were then added, and the mixture well shaken and cooled with water. Enough water was supplied from time to time to dissolve the separated sodium acetate, and two further quantities, each of 5 c.c., of acetic acid and 50 grams of sodium amalgam were subsequently added. When the sodium amalgam had been used up, the liquor was diluted with about 100 c.c. of water, and poured into a hot solution of 7 grams of picric acid in 200 c.c. of water, when on cooling 4(or 5)-γ-aminobutylglyoxaline dipicrate crystallised out. After recrystallisation from water 4·1 grams of the pure salt were obtained, that is, 45 per cent. of the theoretical.

4(or 5)-γ-*Aminobutylglyoxaline dipicrate* crystallises from water in long, golden, somewhat serrated spikes, which melt at 247° (corr.) to a brown liquid, which then decomposes. This salt is anhydrous, and very sparingly soluble in cold, but fairly readily so in hot, water:

0·1163 gave 0·1638 $CO_2$ and 0·0350 $H_2O$. C=38·4; H=3·4.

$C_7H_{13}N_3,(C_6H_3O_7N_3)_2$ requires C=38·2; H=3·2 per cent.

*Reduction of αβ-Bis*[4(or 5)-*glyoxaline*]-*propionitrile.*

Nine grams of αβ-bis[4(or 5)-glyoxaline]-propionitrile hydrogen oxalate* were converted into the base in the manner previously described, and this was reduced by means of 5 grams of sodium

* This vol., p. 677; the formula of the base should read $C_9H_9N_5$.

and 75 c.c. of absolute alcohol. The reaction product was neutralised with hydrochloric acid, made strongly alkaline with sodium carbonate, evaporated to dryness under diminished pressure, and the residue completely extracted with absolute alcohol. After the removal of the bulk of the solvent, the extract was poured into a solution of 20 grams of picric acid in 500 c.c. of boiling water, when a viscous oil separated. After the solution had cooled somewhat it was decanted from the oil and filtered, when it deposited about 3 grams of crystals, melting at 195—200°. The very sparingly soluble oil also became crystalline on cooling, and was extracted with 500 c.c. of boiling water, filtered, and allowed to cool to about 50°, when 5·0 grams of orange needles, melting at 150—153°, separated; these were collected, and the mother liquor allowed to become quite cold, when a further 1·6 grams of crystals, melting at about 210°, separated.

The more sparingly soluble picrate was readily purified by crystallisation from water, and proved to be *βγ-bis*[4(or 5)-*glyoxaline*]-*propylamine tripicrate*; 3·4 grams of this salt were obtained in a pure state, decomposing at 158° (corr.), that is, 18 per cent. of the theoretical. The more easily soluble picrate was less readily purified; it melted and decomposed at 218—220° (corr.) when pure, and was probably *αβ-bis*[4(or 5)-*glyoxaline*]-*propionic acid dipicrate*:

0·1300, air-dried salt, lost 0·0072 at 100°. $H_2O = 5·5$.
$C_9H_{10}O_2N_4,(C_6H_3O_7N_3)_2,2H_2O$ requires $H_2O = 5·1$ per cent.
0·1010 * gave 0·1392 $CO_2$ and 0·0268 $H_2O$. $C = 37·6$; $H = 3·0$.
0·1232 * „ 0·1706 $CO_2$ „ 0·0331 $H_2O$. $C = 37·8$; $H = 3·0$.
$C_9H_{10}O_2N_4,(C_6H_3O_7N_3)_2$ requires $C = 37·9$; $H = 2·4$ per cent.

*βγ-Bis*[4(or 5)-*glyoxaline*]-*propylamine*,
$C_3H_3N_2·CH_2·CH(CH_2·NH_2)·C_3H_3N_2$.

The *tripicrate* crystallises from water in beautiful, orange-yellow, flat needles, which soften from 150° and decompose at 158° (corr.). This salt is sparingly soluble in hot, and very sparingly so in cold, water. It is anhydrous:

0·1330 gave 0·1786 $CO_2$ and 0·0330 $H_2O$. $C = 36·6$; $H = 2·8$.
$C_9H_{13}N_5,(C_6H_3O_7N_3)_3$ requires $C = 36·9$; $H = 2·5$ per cent.

The *trihydrochloride* was prepared by treating the picrate with hydrochloric acid and ether, and after evaporation of the excess of acid was obtained as a colourless varnish. This readily became crystalline when warmed with alcohol. The crystals were collected, dissolved in very little water, and hot alcohol added to the solution,

* Dried at 100°.

when the salt separated in beautiful, colourless, refracting prisms. The air-dried salt contains a molecule of water of crystallisation, which is not lost at 100°, but probably escapes at about 140°, for on heating the salt sinters at this temperature, but then remains unchanged until it melts at 235—237° (corr.). It is readily soluble in water, giving an acid solution, but is insoluble in absolute alcohol:

0·1382 gave 0·1730 $CO_2$ and 0·0730 $H_2O$. C = 34·1; H = 5·9.

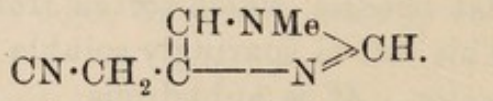
0 1482 „ 0·1981 AgCl. Cl = 33·1.

$C_9H_{13}N_5,3HCl,H_2O$ requires C = 33·9; H = 5·7; Cl = 33·4 per cent.

*Methylation of* 4(or 5)-*Cyanomethylglyoxaline.*

Twenty grams of 4(or 5)-cyanomethylglyoxaline (this vol., p. 676) were dissolved in 80 c.c. of 10 per cent. aqueous sodium hydroxide, and shaken with 16 grams of methyl sulphate added gradually while the liquid was shaken and cooled by running water. Another 70 c.c. of 10 per cent. aqueous sodium hydroxide and 16 grams of methyl sulphate were then added. The clear brown liquor was completely extracted by chloroform, and the solvent removed from the extract, when a brown oil resulted. This was dissolved in water, and poured into 2100 c.c. of warm 2 per cent. picric acid solution, when most of the *1-methyl-4-cyanomethylglyoxaline picrate* crystallised out at once in a pure state. On concentrating the mother liquors, *1-methyl-5-cyanomethylglyoxaline picrate* separated out, mixed with small quantities of its isomeride, and it was purified by recrystallisation from water.

There were isolated 28·5 grams of 1-methyl-4-cyanomethylglyoxaline picrate, melting at 209—210° (corr.), and 9·9 grams of 1-methyl-5-cyanomethylglyoxaline picrate, melting at 156—157° (corr.), these quantities amounting to 43 and 15 per cent. of the theoretical respectively.

1-*Methyl-4-cyanomethylglyoxaline*, 

This base crystallises from chloroform in clusters of plates, which melt at 34—36° (corr.). It is very deliquescent, and readily soluble in water and the usual organic solvents, with the exception of ether and light petroleum:

0·1337 * gave 0·2917 $CO_2$ and 0·0731 $H_2O$. C = 59·5; H = 6·1.

$C_6H_7N_3$ requires C = 59·5; H = 5·8 per cent.

The *hydrogen oxalate* crystallises from water or alcohol in

* Dried at 100°.

prismatic needles, which melt at 116—117° (corr.). It is readily soluble in water, but sparingly so in alcohol:

0·1503 gave 0·2496 $CO_2$ and 0·0572 $H_2O$. C=45·3; H=4·3.

0·1143 „ 19·6 c.c. $N_2$ at 18° and 759 mm. N=20·2.

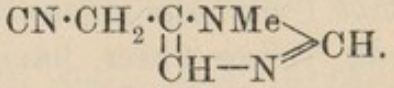
$C_6H_7N_3,C_2H_2O_4$ requires C=45·5; H=4·3; N=19·9 per cent.

The *picrate* crystallises from water in flat, fern-like clusters of stout needles, or in rods of a rather pale yellow colour, which melt at 209—210° (corr.). It is anhydrous, and is very sparingly soluble in cold, but fairly readily so in hot, water:

0·1530 gave 0·2290 $CO_2$ and 0·0395 $H_2O$. C=40·8; H=3·0.

$C_6H_7N_3,C_6H_3O_7N_3$ requires C=41·1; H=2·9 per cent.

The *mercurichloride* and *mercuri-iodide* both readily crystallise from water in long needles.

1-*Methyl-5-cyanomethylglyoxaline*, 

This base was obtained as an oil, which did not crystallise when kept for several hours at 0°. It is readily soluble in water, alcohol, or chloroform.

The *hydrogen oxalate* crystallises from alcohol in prisms, which sinter slightly from 135°, and melt and effervesce at 139—140° (corr.). It is anhydrous. It is readily soluble in water, but sparingly so in alcohol:

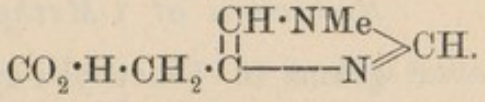
0·1580 gave 0·2632 $CO_2$ and 0·0612 $H_2O$. C=45·4; H=4·3.

$C_6H_7N_3,C_2H_2O_4$ requires C=45·5; H=4·3 per cent.

The *picrate* crystallises from water in large, pale yellow, glistening leaflets, which melt at 156—157° (corr.). This salt is anhydrous, sparingly soluble in cold, but readily so in hot, water:

0·1507 gave 0·2284 $CO_2$ and 0·0411 $H_2O$. C=41·3; H=3·1.

$C_6H_7N_3,C_6H_3O_7N_3$ requires C=41·1; H=2·9 per cent.

1-*Methylglyoxaline-4-acetic Acid*, 

0·75 Gram of 1-methyl-4-cyanomethylglyoxaline was dissolved in 20 c.c. of water, and boiled with 10 c.c. of 10 per cent. aqueous sodium hydroxide until no more ammonia was evolved. Then 9 c.c. of 10 per cent. hydrochloric acid were added, followed by 1·5 grams of picric acid in 40 c.c. of boiling water. On cooling, 1·5 grams of 1-*methylglyoxaline-4-acetic acid picrate* separated in large, striated prisms, melting at 187—189° (corr.), and a further 0·4 gram equally pure was obtained on concentrating the mother liquor, the yield thus amounting to 83 per cent. of the theoretical:

0·1209 gave 0·1751 $CO_2$ and 0·0332 $H_2O$. C = 39·5; H = 3·1.
$C_6H_8O_2N_2,C_6H_3O_7N_3$ requires C = 39·0; H = 3·0 per cent.

*Ethyl* 1-*Methylglyoxaline*-4-*acetate*, $C_4H_5N_2\cdot CH_2\cdot CO_2Et$.

Eight grams of 1-methyl-4-cyanomethylglyoxaline picrate were converted into the base, and this boiled for two hours with 50 c.c. of 15 per cent. absolute alcoholic hydrogen chloride, when ammonium chloride separated. The mixture was evaporated to dryness under diminished pressure, dissolved in water, mixed with sodium carbonate, and extracted with chloroform, when ethyl 1-methylglyoxaline-4-acetate was obtained as a brown oil. This was converted into the picrate, and purified by crystallisation from water, when 4·2 grams of the pure salt were obtained, that is, 46 per cent. of the theoretical.

*Ethyl* 1-*methylglyoxaline*-4-*acetate picrate* crystallises from water in long, yellow fibres, having the appearance of glass wool. It is very sparingly soluble in cold, but readily so in hot, water. It melts at 133—134° (corr.):

0·1092 * gave 0·1700 $CO_2$ and 0·0370 $H_2O$. C = 42·4; H = 3·8.
$C_8H_{12}O_2N_2,C_6H_3O_7N_3$ requires C = 42·3; H = 3·8 per cent.

1-*Methylglyoxaline*-5-*acetic Acid*, $CO_2H\cdot CH_2\cdot C(NMe)=CH-N=CH$.

This compound was prepared by hydrolysis of its nitrile, and isolated as the *picrate*. This salt crystallises from water in beautiful, hexagonal plates, which melt at 180—181° (corr.), after sintering a few degrees earlier. It is anhydrous and sparingly scluble in cold, but readily so in hot, water:

0·1200 gave 0·1732 $CO_2$ and 0·0358 $H_2O$. C = 39·4; H = 3·3.
$C_6H_8O_2N_2,C_6H_3O_7N_3$ requires C = 39·0; H = 3·0 per cent.

*Reduction of* 1-*Methyl*-4-*cyanomethylglyoxaline*.

Seven grams of 1-methyl-4-cyanomethylglyoxaline were reduced by means of 10 grams of sodium and 100 c.c. of absolute alcohol. The reaction product was acidified with hydrochloric acid, made strongly alkaline with sodium carbonate, evaporated to dryness under diminished pressure, and the residue extracted with alcohol. The alcoholic extract was evaporated to dryness, and the residue extracted successively with ether, ethyl acetate, and absolute alcohol.

The ethereal extract amounted to 3·0 grams; it was dissolved

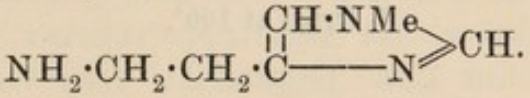
* Dried at 100°.

in water, and poured into a litre of warm 1 per cent. aqueous picric acid, when 6·6 grams of 1-*methyl*-4-*β*-*aminoethylglyoxaline dipicrate* separated in a pure state on cooling. The mother liquor was extracted with ether to remove free picric acid and evaporated to low bulk, when about 0·5 gram of a crude picrate, melting at about 140°, separated. After several crystallisations from water, a very small amount of 1 : 4-dimethylglyoxaline picrate was isolated from it, but was not obtained quite pure. This salt melted at 162—163° (corr.), the pure salt (Trans., 1910, **97**, 1819) melting at 167—168° (corr.) in the same bath, whilst a mixture of the two melted at 162—163° (corr.), and a mixture of this salt with 1 : 5-dimethylglyoxaline picrate at 135—140°.

The ethyl acetate extract afforded a further 1·3 grams of pure 1-methyl-4-*β*-aminoethylglyoxaline dipicrate, so that the total yield of this compound amounted to 7·9 grams, that is, 23 per cent. of the theoretical.

The absolute alcohol extract gave, with picric acid, at first an amorphous precipitate, but later a small quantity of 1-methylglyoxaline-4-acetic acid picrate.

1-*Methyl*-4-*β*-*aminoethylglyoxaline*,



The *dipicrate* crystallises from water in large, flat needles, which melt at 217° (corr.). It is very sparingly soluble in cold water, and is anhydrous:

0·1803 gave 0·2468 $CO_2$ and 0·0498 $H_2O$. C = 37·3; H = 3·1.

$C_6H_{11}N_3,(C_6H_3O_7N_3)_2$ requires C = 37·0; H = 2·9 per cent.

The *dihydrochloride* was prepared from the dipicrate by means of hydrochloric acid and ether. It crystallises from absolute alcohol in colourless prisms, which melt at 204—206° (corr.), after drying at 100°. It is deliquescent, and readily soluble in water, but sparingly so in absolute alcohol:

0·1186 * gave 0·1596 $CO_2$ and 0·0708 $H_2O$. C = 36·7; H = 6·7.

$C_6H_{11}N_3,2HCl$ requires C = 36·4; H = 6·6 per cent.

*Reduction of* 1-*Methyl*-5-*cyanomethylglyoxaline. Formation of* 1-*Methyl*-5-*β*-*aminoethylglyoxaline.*

Three grams of 1-methyl-5-cyanomethylglyoxaline were reduced by means of sodium and alcohol, and the products worked up as in the case of the 1 : 4-compound.

The combined ethereal and ethyl acetate extracts gave first an amorphous picrate, then small quantities of 1-*methyl*-5-*β*-*amino*-

*ethylglyoxaline dipicrate*, which formed yellow needles, melting at 201° (corr.), after drying at 100°:

0·1181 * gave 0·1615 $CO_2$ and 0·0305 $H_2O$. C = 37·3; H = 2·9.

$C_6H_{11}N_3,(C_6H_3O_7N_3)_2$ requires C = 37·0; H = 2·9 per cent.

The mother liquor from this salt then gave a small quantity of 1 : 5-dimethylglyoxaline picrate in clusters of slender needles, melting at 167—168° (corr.):

0·0907 gave 0·1345 $CO_2$ and 0·0267 $H_2O$. C = 40·4; H = 3·3.

$C_5H_8N_2,C_6H_3O_7N_3$ requires C = 40·6; H = 3·4 per cent.

This salt was identified by determination of the melting points of its mixtures with pure 1 : 4- and 1 : 5-dimethylglyoxaline picrates (Trans., 1910, **97**, 1819), when it was found that the mixture with the 1 : 4-salt melted at 135—140°, whilst that with the 1 : 5-salt still melted at 167—168° (corr.).

The alcoholic extract gave a very small quantity of a crystalline picrate which melted at 162—175°; it was probably impure 1-methylglyoxaline-5-acetic acid picrate, but the quantity obtained was insufficient for identification.

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* Dried at 100°.

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