VII. THE SYNTHESIS OF RACEMIC Pr-2-METHYLTRYPTOPHAN.

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(Received March 5th, 1917.)

The mechanism of the changes involved in the complete oxidation of tryptophan in the animal body is quite unknown, partly no doubt on account of the difficulty of synthesising indole derivatives, which might be presumed to be intermediate stages in the degradation of this essential amino-acid. The only known product of its transformation is kynurenic acid which has so far been observed in the dog, the rabbit and the rat. (Note by F. G. Hopkins in a paper by Asayama [1916].) According to Homer [1915] and to Asayama kynurenic acid is not a product on the main line of the destructive breakdown of tryptophan, but in spite of this it is desirable that the mechanism of transformation should become known. "It is possible to picture the conversion of tryptophan to kynurenic acid in many different ways" [Dakin, 1912, p.72]; the first question is whether the single nitrogen atom of kynurenic acid is represented by the indole- or by the amino-nitrogen of its precursor. Ellinger [1904], when he first identified the precursor, accepted the latter alternative, considering tryptophan to be α -amino- α -scatoleacetic acid; kynurenic acid was at that time regarded as being 4-hydroxyquinoline-3-carboxylic acid. The recognition of the true constitution of tryptophan rendered Ellinger's view less likely, until it was shown by Homer [1913] that kynurenic acid is 4-hydroxyquinoline-2-carboxylic acid. This correction of Camps' formula seems to us to re-establish a strong probability in favour of the elimination of the indole-nitrogen, as follows:

On the other hand it has been considered that the pyrrole ring is enlarged to a pyridine ring by the introduction of a carbon atom between the 2 and 3 positions, for which there is a good deal of analogy *in vitro*. Possible mechanisms have been discussed, e.g. by Dakin [1912], but the subsequent recognition of the fact that in kynurenic acid the carboxyl group adjoins the nitrogen atom, has made it necessary to assume, on this hypothesis, that the carboxyl group (oxidised side chain of tryptophan) wanders from the 3-to the 2-carbon atom, which is unlikely.

If by feeding a substituted tryptophan, a substituted kynurenic acid were obtainable, it would be possible to decide between the two hypotheses. A substituent in the benzene ring would be most suitable for this purpose, but is difficult to introduce, and we have therefore contented ourselves for the present with the preparation of a tryptophan methylated at the α -carbon atom of the pyrrole ring (*Pr*-2) which substance is relatively easily synthesised. We were led to this choice by the fact that 2-methylindole (methylketole) is the most readily obtainable indole derivative, and by the observation, that from this substance 2-methylindole-3-aldehyde is obtainable by Gattermann's hydrocyanic acid method in an unexpectedly good yield. From this aldehyde the synthesis followed closely that of tryptophan by Ellinger and Flamand [1907]. The yields were better throughout, probably owing to the protective action of the methyl group on the pyrrole ring, but this very circumstance seems to prevent the rupture of this ring in the animal organism, for, in the only feeding experiment undertaken so far, the urine contained an indole derivative, but neither kynurenic acid nor a similar substance. As we are unable to continue the work at present, we give the details of the synthesis below.

Preparation of 2-methylindole-3-aldehyde.

This substance has been previously obtained by Plancher and Ponti [1907] who acted on 2-methylindole with chloroform and sodium ethoxide, and by Angeli and Marchetti [1907], who obtained it from 2-methylindole and amyl formate, in the presence of sodium ethoxide. We find that Gattermann's aldehyde synthesis is applicable to the present case, and, as in the case of phenols, it is far preferable to the Tiemann-Reimer reaction. 21.5 g. of 2-methylindole was added to 150 cc. of dry ether, 19.2 cc. of anhydrous hydrocyanic acid and 21.5 g. of zinc chloride, freshly fused and powdered; hydrogen chloride was passed in, first for $2\frac{1}{2}$ hours at 0° and finally for $\frac{1}{2}$ hour at 45° . The almost colourless crystalline solid which had separated was filtered off,

washed with ether, boiled for a few minutes with water containing a little ammonia to neutralise traces of adherent acid, and filtered off after cooling. The crude product amounted to 23.9 g., and was somewhat pigmented. After recrystallisation from boiling xylene, in which the pigment is little soluble, we obtained a slightly coloured product melting at 202° ; yield 20 g. or 75 % of the theoretical.

The aldehyde sublimes wholly at 180° in the vacuum of a Gaede pump without melting and when the resulting needles were crystallised from benzene flat acicular prisms were formed, M.P. $202-203^{\circ}$. Like Plancher and Ponti we also observed cube-like crystals.

Azlactone of Pr-3-Methylindolyl- α -benzoylamino-acrylic acid.

17.3 g. of the aldehyde, 22.3 g. hippuric acid, 8.84 g. anhydrous sodium acetate and 39.3 cc. acetic anhydride were heated for 15 minutes on the water bath. The lactone began to separate when the solution was still hot. In a preliminary experiment the reaction product was extracted with boiling water, as indicated by Ellinger, but as we obtained hardly any unchanged aldehyde, we found it simpler to crystallise at once from glacial acetic acid, obtaining thus 30.5 g. of brownish red prismatic plates, melting at $204-207^{\circ}$. Ellinger and Flamand crystallised their lactone from chloroform, of which one molecule was retained as chloroform of crystallisation. Our lactone was not very soluble in chloroform and the air dry crystals retained an indefinite quantity, 10.6 %, rather less than half a molecule. The orange yellow glistening prisms so obtained sintered at 202° , and melted at 211° .

Analysis: 0.1318 dried at 100° ; 0.3666 CO_2 ; 0.0580 H_2O . C = 75.8; H = 4.89.
Calculated for $\text{C}_{19}\text{H}_{14}\text{O}_2\text{N}_2$, C = 75.5, H = 4.63.

Pr-2-Methylindolyl- α -benzoylamino-acrylic acid.

By repeated boiling with 100 times its weight of 1 % sodium hydroxide the lactone was hydrolysed to the above acid. After crystallisation of the precipitated acid from 70 % alcohol we obtained pale yellow prismatic needles, M.P. 221—222°, in a yield of 75 % of the theoretical.

Analysis: 0.1149 dried at 100°; 0.2994 CO₂; 0.0525 H₂O. C = 71.1; H = 5.1.

Calculated for C₁₉H₁₆O₃N₂, C = 71.2, H = 5.0.

Pr-2-Methyltryptophan

4 g. of the above acid were dissolved in 120 cc. of absolute alcohol and heated on the water bath with 12.5 g. of sodium in the course of half an hour. Then 20 cc. of absolute alcohol were added and the solution was boiled for 20 minutes, the flask being immersed in a salt bath. After adding 15 cc. of water, the removal of the benzoyl group was completed by a further boiling for 45 minutes. On pouring into 5 volumes of water the solution was washed with ether, acidified to Congo-red, left in the cold room over night, filtered, washed with ether and ethyl acetate, made up to 5 % with sulphuric acid and precipitated with Hopkins' reagent. After decomposing the mercury precipitate with hydrogen sulphide and treatment with lead carbonate in the usual way, about 60 % of the theoretical yield of an amorphous product was obtained, which yielded sphaerocrystals from 50 % alcohol, but could not be purified by crystallisation.

We therefore treated the concentrated aqueous solution of the substance with a slight excess of warm saturated picric acid solution in water. The gummy picrate which separated, afterwards became partially crystalline. It could be crystallised from picric acid solution (in water it dissociates) but better from methyl alcohol and petrol, which yielded orange red plates, melting at 173°.

Analysis: 0.1000 dried at 100°; 0.1775 CO₂; 0.0364 H₂O. C = 48.4; H = 4.0.

Calculated for C₁₂H₁₄O₂N₂, C₆H₃O₇N₃, C = 48.3, H = 3.8.

Regeneration from the picrate by means of sulphuric acid, washing with ether, and quantitative removal of the sulphuric acid by baryta was not successful. We therefore used the calculated quantity of nitron in dilute acetic acid solution. After filtration from the nitron picrate the solution was evaporated to dryness; the residue was dissolved in a little hot water, boiled with charcoal

and filtered. The amino-acid now crystallised satisfactorily. On recrystallisation from methyl alcohol and ether we obtained colourless prisms, melting variously at 263—273°, according to the rate of heating. The methyltryptophan crystallises with one molecule of methyl alcohol, which is retained *in vacuo* over sulphuric acid, but is given off at 110°.

Analysis:

0.1118 dried at 110°; 0.2861 CO₂; 0.0669 H₂O. C = 65.7; H = 6.25.

Calculated for C₁₂H₁₄O₂N₂, C = 66.05; H = 6.4.

0.1489 dried *in vacuo*, lost at 110° 0.0180 = 12.1 %.

0.0719 dried *in vacuo*, gave 0.1627 CO₂ and 0.0459 H₂O. C = 61.7; H = 7.1. C₁₂H₁₄O₂N₂, CH₃OH requires C = 62.4; H = 7.2; CH₃OH = 12.8 %.

Subsequently we found that the methyltryptophan could be crystallised at once from methyl alcohol and ether, if the crude substance was precipitated a second time by Hopkins' reagent. In this way we obtained a yield corresponding to slightly over 40 % of the theoretical.

Pr-2-Methyltryptophan is readily soluble in water, hardly soluble in absolute ethyl alcohol. It gives a strong reaction with triketohydrindene hydrate. With excess of bromine water a precipitate is produced, but no coloration. Perhaps we may conclude from this, that the bromine reaction of natural tryptophan consists in an attack on the (unsubstituted) α -carbon atom of the indole ring. Methyltryptophan gives with Hopkins and Cole's reagent a greenish blue coloration in dilutions up to 1 : 500. The reaction is therefore very much less sensitive than with the non-methylated amino-acid. In very concentrated solutions the ring test with sulphuric and glyoxylic acids gives at first a reddish purple coloration, which on mixing the two layers becomes deep blue.

The methyltryptophan we obtained has a sweet taste, probably due to its being racemic, for natural *l*-tryptophan is tasteless, *d*-tryptophan is sweet.

Behaviour of methyltryptophan in the dog.

0.5 g. of the substance, dissolved in 10 cc. of water, was given by a stomach tube to a puppy weighing 2 kilos¹. The urine collected during 48 hours after the experiment, was purified by addition of half its volume of 10 % barium chloride containing 5 % ammonia, and kynurenic acid found to be absent,

¹ This experiment was kindly carried out for us by Dr H. H. Dale.

on estimation by Capaldi's method. The urine gave no reaction in the cold with glyoxylic and sulphuric acids, but on heating with 60 % sulphuric acid a dirty green colour was developed, not given by normal urine. We therefore removed the excess of barium, concentrated, acidified and extracted with ether, which solvent did not remove the substance giving the green coloration with hot sulphuric acid. We then evaporated to dryness and extracted the residue with ethyl acetate. The extract contained principally urea, but on destructive distillation of the residue, the vapours gave the pyrrole reaction with pine wood and the residue gave a reddish coloration with picric acid in aqueous solution. It appears possible therefore that methyltryptophan undergoes in the dog a change, which leaves the indole ring intact.

In a subsequent control experiment¹ the puppy was given 0.5 g. tryptophan and yielded 20—30 mg. of kynurenic acid, M.P. 280°.

CONCLUSION.

Further feeding experiments are required, but as long as they are negative it is impossible to draw definite conclusions as to the mechanism of the production of kynurenic acid. We think, however, that on the whole our result is in favour of the view that in natural tryptophan the pyrrole ring is eliminated, and that in methyltryptophan this elimination is prevented by the protective action of the methyl group. If the pyridine ring of kynurenic acid were formed by enlargement of the pyrrole ring, we might expect that methyltryptophan should yield a methylkynurenic acid, just as 2-methylindole yields 3-chloroquinaldine, when acted on by chloroform and alcoholic sodium hydroxide.

We desire to express our indebtedness to Mr W. W. Starling for valuable assistance in the preparation of the compounds described.

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