CXLIV. THE METABOLISM OF TRYPTOPHAN.

II. SYNTHESIS OF 6-METHYL- AND 8-METHYL-KYNURENIC ACIDS.

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THE mechanism of the mode of formation of kynurenic acid from tryptophan in the animal body is not yet fully understood. Of the various ways of attempting the elucidation of this problem it has been suggested that a substituted tryptophan would possibly yield a substituted kynurenic acid, and feeding experiments on these lines were carried out with *Pr*-2-methyltryptophan by Ellinger and Matsuoka [1914] and Barger and Ewins [1917]. In neither case was a substituted kynurenic acid found in the urine of the animal. From the results of their experiments, however, the latter workers concluded that a tryptophan substituted in the benzene nucleus would probably give the desired result. This idea formed the basis of the present investigation.

For reasons advanced in a former paper the author [1924] showed that the most suitable of the many such substituted tryptophans theoretically possible for such a study was *Bz*-3-methyltryptophan. In the same communication the synthesis of this compound was described.

The first problem which presents itself regarding the formation of kynurenic acid is whether its single nitrogen atom is that of the pyrrole nucleus (Theory I), or that of the amino-group (Theory II) of the tryptophan molecule.

If either of these theories is correct, it follows that the feeding of *Bz*-3-methyltryptophan to a dog or to a rabbit should result in the appearance in the urine of the animal of 6-methylkynurenic acid (Theory I) or 8-methylkynurenic acid (Theory II). The isolation, therefore, of either of these acids and its identification by degradation to the corresponding known methylquinoline would, at first sight, appear to accomplish the aim of the investigation. There is, however, a serious drawback to such a mode of procedure. Of the behaviour in the animal organism of compounds of quinoline having structures resembling those of the above methylkynurenic acids little is known. Reference to the literature shows that the only work bearing any relation to this aspect of the investigation was done by Cohn [1895] who studied the fate of certain methylquinolines in the dog and in the rabbit. Of the three methylquinolines which he injected he found that, whereas quinaldine (2-methylquinoline) and *o*-methylquinoline (8-methylquinoline) appeared to be completely destroyed in the animal organism, *p*-methylquinoline (6-methylquinoline) was excreted in the form of 6-carboxyquinoline. The disparity in the behaviour of these compounds made it appear essential that the present investigation should include a study of the fate of the two methylkynurenic acids, which, as shown above, are possible end-products of the metabolism of *Bz*-3-methyltryptophan. For that purpose the synthesis of both these acids was undertaken and is described below.

The schemes of synthesis of 6-methylkynurenic acid and 8-methylkynurenic acid had as their starting-points *p*-toluidine and *o*-toluidine respectively, and

resembled in certain respects that followed by Besthorn [1921] in his synthesis of kynurenic acid from methoxyquinaldine. *p*-Toluidine (1) was condensed with ethyl acetoacetate, the product of this reaction on heating yielded *p*-methyl- γ -hydroxyquinaldine (2). The latter compound readily gave a corresponding chloro-compound (3), which reacted with sodium methoxide to form *p*-methyl- γ -methoxyquinaldine (4).

Condensation of this compound with benzaldehyde resulted in the elimination of water and the formation of benzylidene-*p*-methyl- γ -methoxyquinaldine (5). In his synthesis, Besthorn, for the purpose of introducing a carboxy-group in the β -position (pyridine ring), condensed methoxyquinaldine with formaldehyde, and oxidised the resulting formyl compound with nitric acid. In the present instances it was thought that, under the directive influence of the methyl group, there was a distinct possibility of the use of nitric acid leading to the nitration of the benzene ring. To avoid this, use was accordingly made of potassium permanganate in acetone solution, when a good yield of *p*-methyl- γ -methoxyquinoline-2-carboxylic acid (6) was obtained. Replacement of the methoxy-group in this compound by a hydroxy-group—an operation accomplished by heating with constant-boiling hydrobromic acid—led to the formation of *p*-methyl- γ -hydroxyquinoline-2-carboxylic acid or 6-methylkynurenic acid (7). The constitution of the acid so obtained was established in the following manner. Heating the 6-methylkynurenic acid at its melting-point led to loss of carbon dioxide and the formation of 6-methyl- γ -hydroxyquinoline (8), which compound, on heating with zinc dust, gave 6-methylquinoline (9) which is known in the literature.

The synthesis of 8-methylkynurenic acid followed similar lines.

EXPERIMENTAL.

p-Methyl- γ -hydroxyquinaldine (formula 2). This compound, as well as *o*-methyl- γ -hydroxyquinaldine, was synthesised by Conrad and Limpach [1888] and their method was closely followed. Equimolecular quantities of commercial *p*-toluidine (107 g.) and ethyl acetoacetate (130 g.) were placed in a stoppered reagent bottle and allowed to stand at room temperature for 5 days. An aqueous layer separated and this was removed by distilling the mixture under reduced pressure on the water-bath. The clear oil remaining was transferred to a second distillation flask and rapidly heated to 230°, when the flame was lowered. A violent reaction set in and the temperature rose to 250°. The heating was continued for 10 minutes after the reaction had subsided and the residual highly coloured fluid was quickly poured into a large evaporating basin where it gradually solidified into a dark brown mass. This, when cold, was broken up, boiled with dilute hydrochloric acid and much charcoal and filtered through a hot-water funnel. On cooling, the slightly coloured liquid was neutralised with solid sodium carbonate when a straw coloured crystalline mass separated. This, after standing for 24 hours, was filtered off, dissolved in a large quantity of boiling water to which charcoal had been added, and the

hot solution again filtered. The solution when cold deposited 20 g. of *p*-methyl- γ -hydroxyquinaldine. This was washed with water and dried in the air-oven at 130°. The dry compound melted at 279–280°. (Conrad and Limpach quote *p*-methyl- γ -hydroxyquinaldine as melting at 274–5°.)

o-Methyl- γ -hydroxyquinaldine. The preparation of this compound from *o*-toluidine and ethyl acetoacetate followed exactly the same lines as those described above for the synthesis of its *para*-isomer. The yield, however, was higher, 30 g. being obtained from the same quantities of starting materials. The melting-point of the product was 260–1°, which is that given by Conrad and Limpach.

p-Methyl- γ -chloroquinaldine (formula 3). Following the method of Lieben and Haitinger [1885] who prepared γ -chloropyridine from γ -hydroxypyridine, and that of Conrad and Limpach [1887] for the preparation of γ -chloroquinaldine from γ -hydroxyquinaldine, *p*-methyl- γ -hydroxyquinaldine (12 g.), phosphorus oxychloride (12 g.) and phosphorus pentachloride (14.5 g.) were heated together in a round-bottomed flask at 130°. The preliminary rapid reaction died down almost completely at the end of 30 minutes. On cooling, the liquid set to a semi-crystalline mass. Water was now cautiously added and then a concentrated solution of sodium hydroxide until the contents of the flask were strongly alkaline. The oily mixture was then steam-distilled, when the almost colourless chloro-compound passed over and was apt to solidify in the condenser. When the distillate had stood for 24 hours, the *p*-methyl- γ -chloroquinaldine which had separated was filtered off, dried and purified by distillation under reduced pressure. It distilled at 119°/3.5 mm., the temperature of the bath being 150–3°; yield 9.5 g., M.P. 69–70°. (Found: Cl, 18.25 %. $C_{11}H_{10}NCl$ requires Cl, 18.54 %.)

p-Methyl- γ -methoxyquinaldine (formula 4). *p*-Methyl- γ -chloroquinaldine (15 g.) and sodium methoxide (4.5 g.) dissolved in 10 cc. methyl alcohol were heated together in a bomb tube at 140° for 2 hours. To the mixture was added excess of water with stirring, when fine crystals separated. The mixture was allowed to stand overnight and then filtered. The comparatively pure *p*-methyl- γ -methoxyquinaldine when dry was dissolved in the minimum quantity of hot light petroleum (B.P. 110–20°). From the solution, on cooling, the quinaldine separated as a mass of colourless needles. These were dried on a porous plate: M.P. 106.5–107°, which was not raised by recrystallisation. (Found: N, 7.41 %. $C_{12}H_{13}ON$ requires N, 7.49 %.)

o-Methyl- γ -ethoxyquinaldine (formula 10). For the purpose of protecting the hydroxy-group against oxidation at a later stage of the work, it was replaced, as shown above, by a methoxy group. Substitution of the hydroxy- by an ethoxy-group would serve the same purpose, and, as such substitution can in certain cases be carried out directly by using ethyl-*p*-toluenesulphonate, the action of this reagent on *o*-methyl- γ -hydroxyquinaldine was studied.

o-Methyl- γ -hydroxyquinaldine (12.5 g.) dissolved in absolute alcohol was added to a solution of sodium (1.65 g.) in absolute alcohol. To the mixture was

then added with vigorous shaking ethyl-*p*-toluenesulphonate (14.5 g.) dissolved in a little absolute alcohol. The sodium salt of the toluenesulphonic acid began to separate within a few hours. At the end of 48 hours the mixture was boiled on a sand-bath, cooled and filtered. The excess alcohol was then distilled off from the filtrate, dilute sodium hydroxide added to the residue in the flask and the mixture shaken several times with ether. The combined ethereal extracts were then washed with water and finally with dilute hydrochloric acid. The acid extract so obtained deposited on neutralisation a colourless crystalline precipitate of *o*-methyl- γ -ethoxyquinaldine. This was dissolved in dilute hydrochloric acid. The solution was boiled with a little charcoal and filtered. Addition of alkali reprecipitated the ethoxyquinaldine which was washed several times with distilled water. When dry, it was crystallised from a small quantity of light petroleum. The air-dried crystals melted at 55° and weighed 9.5 g. (65.5 % of theory). (Found: N, 6.90 %. $C_{13}H_{15}ON$ requires N, 6.96 %.)

Benzylidene-p-methyl- γ -methoxyquinaldine (formula 5). *p*-Methyl- γ -methoxyquinaldine (5 g.) and benzaldehyde (4 g.) were heated together in a sealed tube for 6 hours at 175°. The slightly coloured condensation product was dissolved in alcohol, the solution boiled with charcoal and filtered. To the filtrate, while still hot, warm water was added until a cloud began to form, when it was set aside to cool. The benzylidene compound separated in the form of long straw-coloured needles; yield, 6.0 g. (81 % theory); m.p. 140–1°. (Found: N, 5.1 %. $C_{19}H_{17}ON$ requires N, 5.09 %.) It distilled under a reduced pressure (4 mm. Hg) with the metal-bath at 140°. m.p. of the distillate 140–1°. Benzylidene-*p*-methyl- γ -methoxyquinaldine formed a hydrochloride soluble in alcohol and precipitable therefrom by the addition of ether, m.p. 204°.

Benzylidene-o-methyl- γ -ethoxyquinaldine. *o*-Methyl- γ -ethoxyquinaldine (10 g.) and benzaldehyde (5.7 g.) were heated together in a sealed tube for 7 hours at 130–150°, and the resulting condensation product purified according to the procedure described above for the preparation of benzylidene-*p*-methyl- γ -methoxyquinaldine. Benzylidene-*o*-methyl- γ -ethoxyquinaldine was obtained in the form of colourless plates, which melted after repeated crystallisation from dilute alcohol at 115–6°. (Found: N, 4.78 %. $C_{20}H_{19}ON$ requires N, 4.84 %.)

p-Methyl- γ -methoxyquinoline-2-carboxylic acid (formula 6). Benzylidene-*p*-methyl- γ -methoxyquinaldine (7 g.) and potassium permanganate (6.4 g.) were dissolved separately in minimum quantities of purified acetone. When the permanganate was completely dissolved it was added in small portions with mechanical stirring to the solution of the quinaldine compound. The mixture became warm and manganese dioxide slowly separated. When all the permanganate had been added, the mixture was set aside overnight and then filtered. The solid remaining on the Büchner funnel after being washed with acetone was extracted with 400 cc. of hot water three times. The combined aqueous extracts were cooled and neutralised with dilute acetic acid when the

p-methyl- γ -methoxyquinoline-2-carboxylic acid was precipitated. Next morning the precipitate was filtered off and recrystallised from hot water. The purified product melted at 228°. (Found: N, 6.32 %. $C_{12}H_{11}O_3N$ requires N, 6.40 %.)

o-Methyl- γ -ethoxyquinoline-2-carboxylic acid. The method of preparation of this compound from benzylidene-*o*-methyl- γ -ethoxyquinoline by oxidising it in acetone solution with potassium permanganate closely followed that described above for the preparation of *p*-methyl- γ -methoxyquinoline-2-carboxylic acid. Owing to its greater solubility in water than the last-named compound, a slightly different procedure had to be followed to obtain it from the aqueous extracts of the precipitate which separated from the acetone solution during the initial process of oxidation. These extracts, amounting to some 1.2 l., were concentrated on the steam-bath to a small volume (75 cc.). The slightly coloured solution so obtained deposited, on cooling, the potassium salt of the *o*-methyl- γ -ethoxyquinoline-2-carboxylic acid. The potassium salt was very soluble in water and careful neutralisation with acetic acid did not precipitate the free acid. The addition of a concentrated solution of sodium hydroxide to an aqueous solution of the potassium salt precipitated the sodium salt of the acid. The sodium salt thus obtained was purified by dissolving it in the minimum quantity of boiling water and allowing the solution to cool, when it was obtained as a mass of colourless needles. Analysis proved the compound to be pure enough for the purpose of the next experiment. (Found: N, 5.40 %. $C_{13}H_{12}O_3NNa$ requires N, 5.52 %.)

6-Methylkynurenic acid (formula 7). *p*-Methyl- γ -methoxyquinoline-2-carboxylic acid (3 g.) was boiled under reflux with constant-boiling hydrobromic acid (25 cc.) for 4 hours. At first the quinoline compound went into solution but after about 15 minutes the hydrobromide of the methylkynurenic acid commenced to separate. When the reaction was complete, the mixture was allowed to cool. Next morning the almost solid crystalline mass was filtered off. The crystals were then dissolved in the minimum quantity of hot sodium bicarbonate solution and filtered. Careful neutralisation of the warm solution with dilute hydrochloric acid precipitated the 6-methylkynurenic acid in the form of silky needles. The purification of the acid was accomplished by redissolving it in bicarbonate solution, filtering and finally precipitating it with mineral acid. The crystalline mass so obtained was washed with hot water, in which it is insoluble, until the washings were free from chloride.

Analysis: 4.56 mg. gave 10.860 mg. CO_2 , 1.81 mg. H_2O ; C, 64.9 %; H, 4.41 %.

$C_{11}H_9O_3N$ requires C, 65.0 %; H, 4.44 %.

6-Methylkynurenic acid melted at 279° with violent decomposition. Its barium salt forms microscopic needles which are very insoluble in cold water. On long heating of the acid with acetic anhydride in an oil-bath at 140°, a reddish blue colour developed similar to that which can be obtained with kynurenic acid. The coloured compound which separated was slightly soluble

in benzene giving a blue solution. The colour faded on exposing the solution to strong sunlight. Dry 6-methylkynurenic acid heated with hydrochloric acid and potassium chlorate gave a chloro-derivative which, when treated with ammonia, turned mahogany brown, then dark green and finally deep blue (Jaffé's reaction).

The constitution of the new 6-methylkynurenic acid was established in the following manner. 2 g. of the acid in a boiling-tube were heated in a metal-bath at 280–90°. After the evolution of carbon dioxide had ceased, the tube was cooled and the semi-crystalline residue boiled with a little hydrochloric acid. The solution, after being reboiled with charcoal, was filtered and cooled, when the hydrochloride of 6-methyl- γ -hydroxyquinoline crystallised. The crystals were dissolved in aqueous potassium carbonate, and on standing overnight 6-methyl- γ -hydroxyquinoline (formula 8) separated out. Crystallised from hot water and dried at 120°, it melted at 227°.

1 g. of the 6-methyl- γ -hydroxyquinoline obtained in the above manner was ground up with some pure zinc dust, and the mixture heated in a small distillation flask in a metal-bath. The oily distillate which collected in the receiver was dissolved in a little mineral acid, the solution boiled with a little charcoal, filtered and carefully neutralised with alkali. The addition of an equal volume of an aqueous solution of picric acid precipitated a brownish coloured solid which was boiled in turn with water, alcohol, ether and benzene, in all of which it is very insoluble. Dried at 120°, it melted at 227°. (M.P. of 6-methylquinoline picrate as given by Beilstein is 229°.)

8-Methylkynurenic acid. *o*-Methyl- γ -ethoxyquinoline-2-carboxylic acid (3 g.) in the form of its dry sodium salt was boiled under reflux with constant-boiling hydrobromic acid (25 cc.). The solid gradually passed into solution but crystals of the hydrobromide of the 8-methylkynurenic acid did not begin to separate until some 2½ hours had elapsed. After 5 hours' boiling the mixture was allowed to cool and the separated crystals were filtered off next morning and dissolved in dilute sodium bicarbonate solution. This was filtered and hydrochloric acid added to the filtrate as long as any precipitate formed. After 24 hours the precipitate was crystallised from boiling water, in which it was slightly more soluble than the 6-methylkynurenic acid.

8-Methylkynurenic acid melted at 266°. It gave a barium salt which crystallised in the form of small needles slightly soluble in water. The residue obtained by heating it with hydrochloric acid and potassium chlorate to dryness did not give the Jaffé series of colour changes when treated with ammonia, nor did the free acid give a blue compound as was the result when 6-methylkynurenic acid was boiled with acetic anhydride at 140°.

Analysis: 4.800 mg. dried at 100° gave 10.535 mg. CO₂, 2.14 mg. H₂O. C, 59.9 %, H, 5.2 %. C₁₁H₉O₃N, H₂O requires C, 59.7 %, H, 4.98 %.

The constitution of the 8-methylkynurenic acid was established by converting it first into 8-methyl- γ -hydroxyquinoline and then into 8-methyl-

quinoline, the picrate of which substance is already known. 8-Methylkynurenic acid (2.5 g.) was heated at 270–80°. The melt, which remained after the evolution of carbon dioxide had ceased, was cooled and boiled up with dilute hydrochloric acid and charcoal and then filtered. The filtrate, neutralised with potassium carbonate, deposited crystals of 6-methyl- γ -hydroxyquinoline, M.P. 206°. The distillate obtained by heating this compound with zinc dust was boiled with a little hydrochloric acid and charcoal and filtered. The filtrate was carefully neutralised and treated with an aqueous solution of picric acid. The dark brownish precipitate which separated was collected, boiled with water, alcohol and benzene, and dried in the air. The picrate so obtained melted at 196°. (According to Beilstein, 8-methylquinoline picrate melts at 198°.)

SUMMARY.

In order that a study may be made of the behaviour in the animal organism of 6- and 8-methylkynurenic acids, these compounds have been synthesised.

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REFERENCES.

- Barger and Ewins (1917). *Biochem. J.* **11**, 58.
Besthorn (1921). *Ber. deutsch. chem. Ges.* **54**, 1330.
Cohn (1895). *Z. physiol. Chem.* **20**, 210.
Conrad and Limpach (1887). *Ber. deutsch. chem. Ges.* **20**, 952.
—— (1888). *Ber. deutsch. chem. Ges.* **21**, 523.
Ellinger and Matsuoka (1914). *Z. physiol. Chem.* **41**, 45.
Lieben and Haitinger (1885). *Monatsh.* **6**, 315.
Robson (1924). *J. Biol. Chem.* **62**, 495.