CCXXXVII. NOTE ON THE OXIDATION OF TYROSINE, TYRAMINE AND PHENYLALANINE WITH HYDROGEN PEROXIDE.

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(*Received October 22nd, 1932.*)

GUGGENHEIM [1913] has shown that the pods of the broad bean (*Vicia faba*) contain 3:4-dihydroxyphenylalanine, and Miller [1920] has isolated it from the Georgia speckled velvet bean (*Stizolobium deeringianum* Bort). It has also been found in insects [Schmalfuss and Müller, 1927; Przibram, 1922; Schmalfuss, 1927]. Recently, Schmalfuss and Heider [1931] isolated 3:4-dihydroxyphenylethylamine from the pods of the common broom (*Sarothamnus scoparius* Wimm) and found also that it was accompanied by tyramine.

The mode of origin in the plant or animal of these catechol derivatives is unknown, but it does not seem improbable that 3:4-dihydroxyphenylalanine is produced by the oxidation of tyrosine and 3:4-dihydroxyphenylethylamine by the oxidation of tyramine. It might be thought that tyrosinase would be the natural agent to bring about these oxidations, since it has been shown to produce *ortho*-dihydric from monohydric phenols [Pugh and Raper, 1927] and 3:4-dihydroxyphenylalanine has been isolated from the products of its action on tyrosine [Raper, 1926]. On the other hand, tyrosinase acts so rapidly on catechol derivatives that when produced by its action they would not tend to accumulate but be quickly oxidised further. This difficulty concerning their origin in living organisms could be overcome if tyrosinase consisted of two separate enzymes, one of which oxidised monohydric to *ortho*-dihydric phenols and the other oxidised only the *ortho*-dihydric phenols. If the first enzyme could act under conditions unsuitable for the second, then the catechol derivative under these conditions might accumulate. Pugh [1930] has shown, however, that there is no evidence that such a monophenol oxidase exists as a separate component of tyrosinase, and as this is the only enzyme known at present which oxidises monophenolic substances to catechol derivatives it is probable that some other agent is responsible for the oxidation of tyrosine and tyramine to their corresponding 3:4-dihydroxy-compounds.

In the course of experiments on the oxidation of *l*-tyrosine in dilute solution by hydrogen peroxide in the presence of various metallic catalysts it was observed that a trace of ferrous sulphate caused a fairly rapid oxidation of the amino-acid. Examination of the brown solution resulting from the

oxidation showed that it contained a substance giving a marked green colour with ferric chloride changing to reddish purple on the addition of sodium acetate. The substance responsible for this reaction was isolated and found to be *l*-3:4-dihydroxyphenylalanine, identical with the naturally occurring amino-acid. Under similar conditions it has also been found that tyramine is readily oxidised and yields 3:4-dihydroxyphenylethylamine, which was isolated as its tribenzoate.

Dakin and Herter [1907], studying the oxidation of phenylalanine, have shown that the use of hydrogen peroxide under more drastic conditions than were employed in the present experiments causes oxidation in both side-chain and nucleus. It seemed of interest to determine whether under milder conditions of oxidation the side-chain could be kept intact and the aromatic nucleus alone oxidised as with tyrosine and tyramine. To a small extent this has been found possible. By oxidising phenylalanine under the same mild conditions which were used with tyrosine and tyramine a small amount of tyrosine was obtained. This is of interest, since in alcaptonuria homogentisic acid has been shown to be produced from phenylalanine as well as from tyrosine, and Medes [1932] has recently described a condition in which phenylalanine is converted into *p*-hydroxyphenylpyruvic acid *via* tyrosine.

Several attempts have been made by the author to obtain homogentisic acid by the oxidation of *p*-hydroxyphenylpyruvic acid by hydrogen peroxide and iron, but they have all been unsuccessful.

These results show, therefore, that a possible mode of origin of *l*-3:4-dihydroxyphenylalanine and its corresponding base may be the oxidation of *l*-tyrosine and tyramine respectively by hydrogen peroxide under the catalytic influence of iron. They also demonstrate that tyrosine may be produced by the direct oxidation of phenylalanine and they add a few examples to the many which already exist of the probable importance of hydrogen peroxide as an oxidising agent in living organisms.

Experimental.

Oxidation of tyrosine.

To a solution of 2 g. tyrosine in 4 litres of water at room temperature were added 0·1 g. $FeSO_4, 7H_2O$ and 10 cc. (1·5 mols.) of 6 % H_2O_2. The solution soon became yellow and then gradually darkened to a brownish colour, depositing a little dark brown pigment. After standing for 3 days the solution was filtered and 10 g. lead acetate in 50 cc. water were added to the filtrate. This precipitates most of the brown pigment. The precipitate was filtered off and rejected. The filtrate on making definitely alkaline with ammonia gave a bulky precipitate. This was filtered off, washed a few times with cold water and then, after suspending in 200 cc. water, decomposed with H_2S. After removing the lead sulphide the solution was concentrated by distillation under reduced pressure until a good crystalline deposit had separated. The solution

was then heated to dissolve the deposit, transferred to a beaker, allowed to cool, neutralised to Congo red and concentrated to about 15 cc. in a vacuum desiccator over sulphuric acid. 0·32 g. of crystalline 3:4-dihydroxyphenylalanine was thus obtained; M.P. 275–80° (decomp.). By evaporating the mother-liquor to very small bulk a further crop of crystals of the amino-acid separated. In all 0·58 g. of crude dihydroxyphenylalanine was thus obtained. One recrystallisation from boiling water containing a trace of acetic and sulphurous acids gave a product with M.P. 279–80° (decomp.). This is the M.P. of the laevo-form of the amino-acid. It was greenish in colour due to traces of iron which are not completely removed in the process of separation *via* the lead salt. Attempts to remove the iron by decomposing the lead salt in presence of enough ammonia to render the solution alkaline resulted in a colourless product but with a lower M.P. 269–70°, this being due to racemisation. A sample of the racemised amino-acid obtained in this way, M.P. 268–9° (decomp.) was analysed: C, 55·2; H, 5·9; N, 7·1 %. $C_9H_{11}O_4N$ requires C, 54·8; H, 5·6; N, 7·1 %. By recrystallising the product of M.P. 279–80° three times from weakly acidulated water as above, crystals were obtained with only a slight colour. These were examined polarimetrically. 0·15 g. in 3·05 cc. *N* HCl gave $[\alpha]^{18°}_{5461} = -13·6°$. This is the value found by Harington and Randall [1931] for pure *l*-3:4-dihydroxyphenylalanine.

The amino-acid reduced ammoniacal silver nitrate in the cold and gave an intense green colour with ferric chloride.

Oxidation of phenylalanine.

3 g. inactive phenylalanine were dissolved in 3 litres of water and 0·15 g. $FeSO_4, 7H_2O$ added. 15 cc. of 6 % hydrogen peroxide were then introduced and the solution kept at room temperature until, after 4 days, the hydrogen peroxide had disappeared (chromic acid test). The solution had become deep brown and there was a brownish-black deposit. It was filtered, concentrated by distillation under reduced pressure to about 500 cc. and then 80 cc. of 20 % lead acetate were added together with 2*N* ammonia until no further precipitate came down. The lead precipitate was filtered off, washed and decomposed with H_2S in 600 cc. water. The PbS was removed by filtration, well washed and the filtrate and washings concentrated by distillation under reduced pressure to about 50 cc. and then, after neutralising to Congo red with ammonia, in a vacuum desiccator. A crystalline deposit was obtained which was recrystallised from boiling water, clarifying with charcoal. The substance crystallised in fine needles exactly like tyrosine; M.P. 287° (decomp.). Analysis, C, 59·3; H, 5·5; N, 7·6 %. $C_9H_{11}O_3N$ requires C, 59·7; H, 6·1; N, 7·7 %. The product was readily oxidised by tyrosinase from mealworms giving the typical colour changes of tyrosine. It also gave Millon's reaction. *o*- and *m*-Tyrosine both have a lower M.P. than that of the oxidation product and are not oxidised by tyrosinase from mealworms [Abderhalden, 1928]. From 4 g. of phenylalanine 167 mg. of the crude and only 43 mg. of the recrystallised tyrosine, M.P. 287°,

were obtained. The mother-liquor from which the first crop of tyrosine was obtained gave a further crop of crystals on concentration consisting largely of tyrosine as judged by the M.P. (270° after recrystallisation). The final mother-liquor gave a reddish-violet colour with ferric chloride suggesting the presence of *o*-tyrosine, but this was not confirmed in a second experiment. It is doubtful therefore whether *o*-tyrosine is produced along with the relatively more abundant *p*-tyrosine.

Oxidation of tyramine.

To 2 g. tyramine hydrochloride in 2 litres of water 0·05 g. $FeSO_4,7H_2O$ was added and then 60 cc. of 1 % H_2O_2 (1·5 mol.). The solution was allowed to stand overnight at room temperature, after which the H_2O_2 had disappeared. It was brownish-coloured and gave a marked catechol reaction with ferric chloride. 10 cc. of sulphurous acid were added and the solution was concentrated by vacuum distillation to about 100 cc. 50 cc. of 20 % lead acetate were added and then 2*N* ammonia until no further precipitate was formed. The lead precipitate was filtered off by suction on a pulp filter, washed with a little water and the paper pulp and precipitate suspended in 400 cc. water. The lead compound was now decomposed with H_2S, the lead sulphide and pulp removed by filtration and washed with hot water. The filtrate and washings were concentrated by vacuum distillation to about 30 cc. and then, after addition of 1·5 cc. concentrated HCl, in a vacuum desiccator. The dark brown crystalline residue was extracted with 20 cc. hot alcoholic HCl, filtered and the filtrate concentrated in a vacuum desiccator. A small amount of a sticky brown substance separated and was filtered off. The filtrate was now taken nearly to dryness in the desiccator when a brownish crystalline mass separated. This was filtered off, washed with a little absolute alcohol and dried *in vacuo*; 0·45 g. was obtained. It gave a strong catechol reaction with ferric chloride and was presumably the crude hydrochloride of 3:4-dihydroxyphenylethylamine. It was dissolved in a few cc. of water and benzoylated, using 5 g. benzoyl chloride and 30 cc. 10 % NaOH. The benzoylated base which separated was shaken out with benzene. On distilling off the benzene a viscous residue remained. This was redissolved in 5 cc. benzene and allowed to stand overnight in order to remove any tyramine dibenzoate, which is only slightly soluble in benzene. None was obtained. The solution was now allowed to evaporate slowly until crystals appeared. The crystallisation was aided by the cautious addition of light petroleum. The crystals thus obtained were filtered off, washed with a mixture of 3 parts benzene and 1 part light petroleum and dried *in vacuo*; M.P. 136–7°. On recrystallisation twice from 80 % aqueous alcohol and again from benzene and light petroleum a product was obtained with M.P. 140–1°. This is the M.P. of the tribenzoate of 3:4-dihydroxyphenylethylamine [Schmallfuss and Heider, 1931]. Micro-analysis, C, 74·6; H, 5·15; N, 3·2 %; $C_{29}H_{23}O_5N$ requires C, 74·8; H, 4·9; N, 3·0 %.

SUMMARY.

By the action of hydrogen peroxide with ferrous sulphate as a catalyst it is shown that phenylalanine yields tyrosine, *l*-tyrosine yields *l*-3:4-dihydroxyphenylalanine and tyramine yields 3:4-dihydroxyphenylethylamine.

A grant in aid of this investigation from the Government Grant Committee of the Royal Society is gratefully acknowledged.

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