RESEARCHES ON AMINES.

X. THE FORMATION OF TYRAMINE BY DECARBOXYLATION OF TYROSINE PRODUCED FROM SILK.*

By TREAT B. JOHNSON and P. G. DASCHAVSKY.†

(*From the Department of Chemistry, Yale University, New Haven.*)

(Received for publication, November 10, 1924.)

The first chemical method for the production of tyramine II was that of Schmitt and Nasse,[1] who obtained it by heating small quantities of tyrosine I to a temperature of 270°. The yield obtained by this method was very poor. Ehrlich and Pistschimuka,[2] in a later study of the thermal decomposition of tyrosine, attempted to improve the yield of tryamine II by heating the amino acid slowly at 270° under a

$$\underset{\text{I}}{HO\cdot C_6H_4CH_2CH(NH_2)COOH} \longrightarrow CO_2 + \underset{\text{II}}{HO\cdot C_6H_4CH_2CH_2NH_2}$$

pressure of 12 to 25 mm. The tyramine was formed as a sublimate and recovered in the form of its hydrochloride. The application of their method is limited to decomposition of small quantities of tyrosine (1 gm.) and the yield of tyramine is about 50 per cent of the theoretical.

This same decomposition of tyrosine can also be accomplished by biochemical methods. It is produced from tyrosine in the putrefaction of proteins containing this amino acid, but the amount formed in such cases is usually small and the methods of separating and isolating the base in a pure condition are lengthy and cum-

* Constructed from part of a dissertation presented by P. G. Daschavsky to the Faculty of the Graduate School of Yale University, June, 1920, in candidacy for the degree of Doctor of Philosophy.

† Holder of the du Pont Fellowship in Chemistry, 1918–19, and the Loomis Fellowship in Chemistry, 1919–20. Deceased, July 21, 1924.

[1] Schmitt, R., and Nasse, O., *Ann. Chem.*, 1865, cxxxiii, 211.

[2] Ehrlich, F., and Pistschimuka, P., *Ber. chem. Ges.*, 1912, xlv, 1006.

bersome. Sasaki[3] showed that tyrosine can be decarboxylated by the action of *Bacillus coli communis*. The essential features of his procedure are the use of tyrosine and a suitable nutrient medium prepared under sterile conditions to which are added agar-agar colonies of the bacterial organism, freshly isolated from feces and cultivated for 24 hours on agar-agar, and incubated at 37° for 40 days. The tyramine is extracted by means of alcohol and ether and finally purified in the form of its hydrochloride. The yield reported by application of this method with 10 gm. of tyrosine is 78.7 per cent of the theoretical.

Later, Kawai[4] patented a biochemical process for decarboxylation of tyrosine. In his process he used the bacterium *Bacillus proteus vulgaris* to decompose the amino acid and incubated it for a long time at 37° to obtain complete transformation of the tyrosine to tyramine. As the original Japanese patent literature was not available to us and the abstract was very abbreviated we were unable to obtain the exact details of his method of operating. Apparently his procedure has no advantage over that recommended by Sasaki; also it is impossible even to approximate the cost of production by either method.[5]

The method of decarboxylation that is of immediate interest to us in our work is that devised by Graziani.[6] This investigator, in an attempt to synthesize cyclo-tyrosyl-tyrosine by heating tyrosine with diphenylmethane, made the very interesting observation that the amino acid was decarboxylated under these conditions and tyramine was produced. Graziani states that his best yield of tyramine was 97 per cent of the theoretical and was obtained by heating a mixture of 1 gm. of tyrosine and 15 cc. of diphenylmethane at 245° for 2½ hours. The tyramine was easily separated after completion of the reaction, and crystallized on cooling, being finally purified by distillation under diminished pressure.

[3] Sasaki, T., *Biochem. Z.*, 1914, lix, 429; *J. Biol. Chem.*, 1917, xxxii, 527. Sasaki, T., and Otsuka, I., *J. Biol. Chem.*, 1917, xxxii, 533.

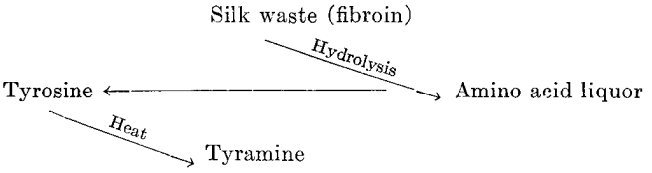
[4] Kawai, K., Japanese Patent, No. 30,334, November 14, 1916; *Chem. Abstr.*, 1917, xi, 2027.

[5] See also Hanke, M. T., and Koessler, K. K., *J. Biol. Chem.*, 1922, l, 271; Bettinger, *Bull. assn. chim. sucr. dist.*, 1921, xxxviii, 463; *Chem. Abstr.*, 1922, xvi, 1104.

[6] Graziani, F., *Atti. accad. Lincei*, 1915, xxiv, series 5, pt. 1, 822, 936.

A repetition of Graziani's work by the writers has led to the confirmation of his results. However, if 10 gm. units of tyrosine are used instead of 1 gm., as utilized by this investigator, the decarboxylation period is much longer, 8 to 10 hours being required to complete the reaction. For large scale production this method lacks the proper details and, furthermore, its practical application is dependent on the availability of tyrosine in quantity. His paper, however, furnished us the clue for a new method of operating which has made tyramine a reagent which can now be obtained easily in a pure condition for experimental purposes.

The method which we have developed for the preparation of this interesting base is represented by the scheme given below and is based on the utilization of waste silk (crude fibroin) as a source of the amino acid, tyrosine. Silk gum or sericin, which is also a waste product in the silk industry, is not a suitable protein for tyramine manufacture on account of its low content of tyrosine.



For obtaining the maximum yield of tyrosine from fibroin, Fischer and Skita[7] recommend the procedure of Weyl,[8] using 25 per cent sulfuric acid as the hydrolyzing agent. This method we have adopted and it possesses one great advantage over that by hydrochloric acid, in that the acid can be subsequently easily and completely removed by precipitation as barium sulfate. In considering this well known laboratory procedure, it was found necessary, in order to make the method one of practical utility in our work, to substitute another alkali for barium hydroxide, and thereby eliminate the use of carbon dioxide for precipitating barium as carbonate, and finally to displace oil baths and direct heat in operating the digestion or hydrolysis of the protein. These factors of technical improvement have been accomplished as follows: (1) by passing steam through a lead coil immersed in the

[7] Fischer, E., and Skita, A., *Z. physiol. Chem.*, 1901, xxxiii, 177.
[8] Weyl, T., *Ber. chem. Ges.*, 1888, xxi, 1529.

acid-protein solution as the method of heating during hydrolysis; (2) by using white lime as the reagent to neutralize sulfuric acid instead of barium hydroxide; and (3) by eliminating the use of carbon dioxide, merely by the reprecipitation and recrystallization of the crude tyrosine.

Decarboxylation of Tyrosine.—While the degradation of α-amino acids to their corresponding amines by bacterial or fungicidal action is well understood and suitable organisms for bringing about this change have been isolated and described, the literature on chemical methods for accomplishing this transformation is very scanty. Cahours[9] distilled the simpler α-amino acids with lime and baryta and obtained the corresponding amines, but no quantitative data of value are recorded in his publication. Both Schwanert [10] and Limpricht[11] merely heated the α-amino acids to obtain the corresponding amines, but here again no data are given regarding yields.

As a general rule dry distillation methods are not productive of good yields, especially when one works with easily carbonized materials. In the specific case of tyrosine, heating the acid alone produces very little tyramine. In heating a crystalline substance of this character, the heat is unevenly distributed so that at one spot, carbonization may take place, while at another, the temperature may be too low to cause decarboxylation. This very problem arose when different investigators tried to make the anhydrides of certain amino acids by heating the solid material. Maillard[12] employed glycerol to moderate the reaction and to increase the yield of degradation products. This procedure proved successful and Maillard assigned this specific effect to the glycerol. Balbiano[13] concludes, on the other hand, that glycerol exerts no specific action in this change and that anhydride formation is due only to the high temperature used. The glycerol only acts as a diluent and conducts the heat, and, furthermore, may be replaced by other material such as hydrocarbons. Bal-

[9] Cahours, A., *Ann. Chem.*, 1859, cix, 29.
[10] Schwanert, H., *Ann. Chem.*, 1857, cii, 225.
[11] Limpricht, H., *Ann. Chem.*, 1857, ci, 297.
[12] Maillard, L. C., Genese des matieres proteiques et des matieres humiques, Paris, 1913; *Ann. chim.*, 1914, i, 519; *Chem. Abstr.*, 1914, viii, 1594, 3423.
[13] Balbiano, L., *Atti accad. Lincei*, 1914, xxiii, series 5, pt. 1, 893.

biano showed that naphthalene and cymene can be utilized successfully in bringing about anhydride formation.

Graziani[9] confirmed Balbiano's work, extending the observations to diphenylmethane and acenaphthene. In the cases of alanine, leucine, and tyrosine he obtained a smooth conversion to the corresponding amines by heating in the presence of diphenylmethane. Graziani concludes that for each amino acid there is a lower temperature at which anhydrification will be at a maximum, and a correspondingly higher temperature at which, instead, the formation of amines will be at a maximum, but between these two temperatures both reactions are simultaneously possible. It is evident, therefore, that when a given solvent facilitates one type of reaction this will predominate even at a temperature beyond its limits. Thus, glycerol has a great avidity for water and consequently, as a solvent, favors anhydride formation. In general, it may be said, therefore, that the α-amino acid grouping III will evolve carbon dioxide on heating,

$$\underset{\text{III}}{\text{R}\cdot\text{CH}(\text{NH}_2)\text{COOH}} \longrightarrow \text{R}\cdot\text{CH}_2\text{NH}_2 + \text{CO}_2$$

substituent groupings (R), of course, influencing the reaction. The yield of resulting amine, however, is a variable factor and is dependent on heat distribution, side reactions, and stability of the amino acid and amine.

After a great many experiments to determine the best solvent for promoting or catalyzing the dissociation of tyrosine into carbon dioxide and tyramine, we have finally adopted a mixture of equal parts of diphenylmethane and diphenylamine. Most excellent results have been obtained by its use and the mixture possesses the following advantages. (1) It remains fluid when cooled to 0°; (2) it has a high boiling point, 260–300°, at atmospheric pressure; (3) the tyramine formed in the reaction is soluble in the hot, but insoluble in the cold solvent, and consequently the latter may be separated from the tyramine by filtration and washing with benzene; (4) the mixture of hydrocarbon and amine is not an expensive reagent to use and the recovery is large; and (5) this special solvent regulates the decarboxylation reaction to give an almost quantitative yield of tyramine (95 to 97 per

cent) in a very short period of heating and with minimum carbonization.

To summarize, we have here the two essential features of a practical method for the production of tyramine from tyrosine; namely, a commercial waste product suitable for the production of tyrosine, and an easy and practical method of converting the latter into tyramine. The success of our method of operating is evidenced by the result obtained in a single experiment when we obtained from 2,000 gm. of silk noils (crude fibroin) 94 gm. of pure, colorless tyramine hydrochloride.

EXPERIMENTAL PART.

Hydrolysis of Fibroin.—For the preparation of tyrosine from fibroin we have, in agreement with Fischer, found that 20 to 25 per cent sulfuric acid is a very suitable reagent for hydrolyzing the protein. The hydrolysis was carried out in a lead-lined vessel of about 40 liters capacity, and the heating done by means of an immersed lead coil, through which steam circulated. This vessel was covered by a lead top containing openings for the ends of the immersed coil and a small aperture for sampling purposes.[14] To explain the details of our method of operating we will give a description of a single experiment.

Composition of Mixture.

Silk noils (crude fibroin)	2,000 gm.
Concentrated H_2SO_4	4,000 cc.
Water	20,000 "

Procedure.

The 20 per cent H_2SO_4 was poured into the kettle with the lead coil in position, and the silk noils were added in portions of 200 gm. Care must be taken to insure thorough wetting of the noils by the acid. The lead cover was then placed in position and steam allowed to circulate through the coil for about 60 hours. Small samples of the material were withdrawn from time to time and subjected to the biuret test, which was used as a criterion for judging the completeness of hydrolysis of the protein. When the

[14] All the silk noils (crude fibroin) used in this investigation were furnished gratuitously by Cheney Bros., Silk Manufacturers of South Manchester, Conn.

mixture no longer gave the biuret coloration, the heating was stopped and 20 liters of water were added. The diluted acid liquor was then neutralized with finely powdered lime, using a motor-driven stirrer to agitate the contents of the kettle, the neutral mass filtered by suction on large porcelain suction filters, and the precipitate of $CaSO_4$ washed with 10 liters of hot water. After removing the $CaSO_4$ from the filter, it was then digested with 90 liters of boiling water in six portions, using the motor stirrer for agitation, and at the same time blowing steam through the mass, then allowed to settle, and filtered. After careful washing of the $CaSO_4$ with hot water the combined filtrates were added to the original mother liquor and the solution was evaporated in an enameled pan to a volume of about 15 liters. At this point, the liquid was again tested for neutrality and allowed to stand overnight. Tyrosine which separated was filtered and washed with cold water. The filtrate at this point contains the residual amino acids of the protein and is saved.[15]

The tyrosine which separates on standing contains some $CaSO_4$ as impurity, and to purify the acid it was digested on the steam bath successively with two portions of an alkaline solution containing 500 gm. of NaOH in 2 liters of water. This treatment served to dissolve the tyrosine away from the lime impurities. The alkaline liquor was then filtered by suction, washed with water, the filtrate diluted with a liter of water, and neutralized exactly with hydrochloric acid, using Congo red as an outside indicator. At the neutral point tyrosine precipitated and was filtered by suction and washed thoroughly with cold water. To obtain the amino acid in a colorless and pure condition, the crude material was dissolved in 20 liters of boiling water containing some "norit" as decolorizing agent, filtered hot, and allowed to crystallize. The pure tyrosine was then filtered, washed with cold water, and dried at 100°. From 2,000 gm. of fibroin we obtained 140 gm. of the pure amino acid, equivalent to a yield of 7 per cent. Many hydrolysis experiments with varying amounts of noils confirmed this result.

The silk noils (fibroin) used in our research were derived from Chinese Canton silk, and the yield of tyrosine obtained (7 per cent) compares very favorably with the quantitative result (9.8 per cent) as recorded by Plimmer[16], and expressed in Table I.

Conversion of Tyrosine into Tyramine.—The basis for the following experimental development is the interesting result obtained by Graziani in which tyramine is produced from tyrosine by heating with diphenylmethane. Of the various organic solvents tested for their power of decarboxylating this amino acid, an equal mix-

[15] Uses for this valuable amino acid liquor are being developed (T. B. Johnson).

[16] Plimmer, R. H. A., Chemical constitution of the proteins, Monographs on biochemistry, London, New York, Bombay, and Calcutta, 2nd edition, 1912–13.

ture of diphenylamine and diphenylmethane was found to give the most satisfactory results. A description of a single experiment will illustrate the technique of our method of operating.

20 gm. of tyrosine were mixed with 120 cc. of pure distilled diphenylmethane and 120 gm. of diphenylamine in a liter round bottom Pyrex flask, fitted with an air condenser, and the mixture was carefully heated with a Bunsen flame, care being taken to prevent local superheating or charring. At 260° carbon dioxide began to be liberated and in a few minutes the evolution of this gas was copious; and at the end of 40 minutes heating at a temperature of 260–265°, the reaction was apparently completed, the hydrocarbon-amine solution presenting a clear yellow appearance without any indication of carbonization. Although the melting point of tyrosine is about 295° our decarboxylation had been effected in this medium at 260°. The clear yellow solution was allowed to cool to 60° when the mass became

TABLE I.

Yield of Tyrosine by Hydrolysis of Fibroin from Different Sources.

Silk fibroin.	Tyrosine.
	per cent
Italian	10.5
Chinese, New Chwang	9.8
" Canton	9.8
" Shantung	9.7
" Niet-ngo-Tsam	7.8
" Tai-Tsao-Tsam	7.8
" Cheefoo	8.5
" Tailung	3.6
Indian, Bengal	10.0
"	9.2

quite cloudy. At this point 100 cc. of benzene were added to prevent tyramine from adhering to the walls of the flask, and the contents agitated to cause thorough mixing of the benzene. 1 hour cooling in an ice bath caused the tyramine to deposit as a fine yellow powder which was filtered off by suction and washed several times with warm benzene to remove the solvent mixture. What tyramine adhered to the walls of the reaction flask was recovered by conversion to the hydrochloride and extracted with water.

From 140 gm. of tyrosine thus treated in seven runs of 20 gm. each, there were obtained 86 gm. of crude tyramine and 18.5 gm. of crude tyramine hydrochloride, equivalent to 14.6 gm. of the base, giving a total of 100.6 gm. of crude tyramine. A quantitative yield of tyramine from tyrosine is 105.98 gm. Therefore, our

yield of tyramine is equivalent to 95 per cent of the theoretical, a result which establishes this method of preparation as the most productive and practical of any hitherto described.

The crude tyramine was digested with 300 cc. of concentrated hydrochloric acid and 600 cc. of water, the solution filtered and then concentrated by evaporation *in vacuo* on the steam bath to incipient crystallization, and then quickly poured into a beaker and allowed to crystallize. A second crop of the hydrochloride was obtained by further concentration of the acid liquor. The hydrochloride was finally purified by recrystallization from hot absolute alcohol, strongly acidified with hydrochloric acid gas, when it separated, on cooling, as colorless glistening needles. Three crops of the hydrochloride were collected as follows:

1st	66 gm.	melting at	269–270°
2nd	20 "	" "	268.5–269.5°
3rd	8 "	" "	267.5–268°
Total	94 "	tyramine hydrochloride.	

A portion of the first fraction was again crystallized from absolute alcohol containing hydrochloric acid and, when dried *in vacuo* over concentrated sulfuric acid for 48 hours, had the following properties.

It melted at 269.5–270° to a clear liquid. Koessler and Hanke[17] give 280° as the melting point of their tyramine hydrochloride containing 1.86 per cent of sodium chloride as impurity. Barger[18] reports a melting point of 268°. Analysis: 0.3152 gm. of tyramine hydrochloride ignited in a weighed platinum crucible at a red heat left no weighable residue. 0.4860 gm. of tyramine hydrochloride when treated in aqueous solution with $AgNO_3$ gave 0.4001 gm. of AgCl instead of the theoretical amount or 0.4007 gm. Nitrogen determination (Kjeldahl method):

$C_8H_{11}ON \cdot HCl$. Calculated. N 8.07.
Found. " 8.00, 8.01.

Recovery of Solvents.—The benzene used for washing the tyramine can easily be separated from the solvent mixture by dis-

[17] Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.*, 1919, xxxix, 585.

[18] Barger, G., The simpler natural bases, Monographs on biochemistry, London, New York, Bombay, and Calcutta, 1914, 10, 18; *J. Chem. Soc.*, 1909, xcv, 1123; English Patent, No. 1,560, 1909. Barger, G., and Walpole, G. S., *J. Chem. Soc.*, 1909, xcv, 1720; English Patents, Nos. 1,561 and 17,171, 1909.

tillation under diminished pressure. The resulting liquid consisting of diphenylamine and diphenylmethane, when fractionally distilled *in vacuo*, is easily purified for further work, giving a satisfactory and clean-cut separation.

Rejected Solvents.—In testing the various solvents for decarboxylating tyrosine, a specially constructed apparatus was employed which enabled us to determine quantitatively the amount of CO_2 generated from a known quantity of tyrosine. By weighing the evolved CO_2 a relative measure of the catalytic influence of solvents tested could be determined without isolating the tyramine formed. The solvents tested were paraffin, white vaseline, aniline, quinoline, α-naphthylamine, anthracene, diphenylmethane, diphenylamine, and diethylaniline.

Paraffin was found to promote decomposition of tyramine, but it was very difficult to separate the base from this solvent. Vaseline was also rejected for the same reasons. Aniline, which allowed of heating only to 183°, did not cause decarboxylation of the amino acid. Quinoline when heated to its boiling point dissolved tyrosine quickly. However, the tyramine, if formed, does not crystallize on cooling and separation of quinoline and tyramine is not accomplished easily.

Diethylaniline did not convert tyrosine to tyramine in any satisfactory manner under the maximum temperature conditions; namely, 216°. A temperature of about 250–260° was found to be necessary to decarboxylate the tyrosine, and an inspection of the boiling points of aniline, quinoline, and diethylaniline shows them to lie below the minimum temperature required for decomposing the amino acid. The use of α-naphthylamine was prohibited because of the tar formed and the difficulty in isolating the tyramine formed. In fact, no pure tyramine could be isolated. Anthracene was found to be valueless as a solvent because of its high melting point (217°) and great tendency to sublime.

SUMMARY.

1. A practical method has been developed for the production of the important, active amine, tyramine, from silk fibroin.

2. The method developed has several advantages over those previously described in the literature, and is based on the well known behavior of tyrosine when heated above 260°.

$$HOC_6H_4CH_2CH(NH_2)COOH \rightarrow CO_2 + OH \cdot C_6H_4CH_2CH_2NH_2$$

3. Utilization is made of a trade waste product (silk noils), and the complete synthesis is accomplished in two major operations; namely, (*a*) hydrolysis of silk fibroin to produce the amino acid, tyrosine, and (*b*) decarboxylation of the tyrosine to tyramine by heating at 260° in a catalytic medium composed of equal parts of diphenylmethane and diphenylamine.

4. This investigation has revealed the importance of a more thorough study of catalytic reagents which facilitate the formation of amines by heating amino acids.