[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, LOS ANGELES]

The Biogenesis of Nicotine and Anabasine[1]

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The metabolism of lysine-2-C^{14} in nicotine producing *Nicotiana tabacum* and in anabasine producing *Nicotiana glauca* has been studied. The nicotine isolated from the plant was inactive, but the anabasine was radioactive. Systematic degradation of the anabasine (2-(3-pyridyl)-piperidine) indicated that all the radioactivity resided in the α-carbon atom of the piperidine ring attached to the pyridine ring. The significance of these results is discussed.

Introduction

The synthesis of nicotine in the tobacco plant has attracted the attention of the plant physiologist and the organic chemist.[2] Recent feeding experiments with isotopically labeled compounds have indicated that the N-methyl group of nicotine arises by transmethylation from methionine[3,4] or choline.[5] Glycine-2-C^{14},[6] serine-2-C^{14},[7] formaldehyde-C^{14}[7] and calcium glycolate[8] labeled in the α-position with C^{14}, all gave radioactive nicotine with a majority of activity present in the N-methyl group. It has been reported[9] that excised tobacco leaves exposed to radioactive carbon dioxide gave nicotine labeled in the N-methyl group; however, the feeding of C^{14}-labeled sodium bicarbonate to intact tobacco plants resulted in over-all labeling of the nicotine molecule with a slight preferential incorporation of active carbon into the N-methyl group.[10]

Until recently little has been known about the mode of formation of the carbon skeleton of nicotine. Administration of proline, pyrrolidone carboxylic acid or nicotinic acid appeared to increase the amount of nicotine in the plant.[11,12] However the feeding of nicotinic acid or its ethyl ester labeled in the carboxy group with C^{14} to excised tobacco roots growing in sterile culture failed to yield any radioactive nicotine.[13] Mortimer[14] suggested that nicotine could be derived from metabolites of tryptophan but this was rendered improbable by the feeding of tryptophan-2-C^{14} to intact tobacco plants when inactive nicotine was obtained.[15] It has been shown by the author[16] and independently by Byerrum, *et al.*,[17] that ornithine is a precursor of the pyrrolidine ring of nicotine.

There have been many biogenetic schemes for the formation of nicotine and one of the first was that of Robinson[18] who suggested that the alkaloid arose from ornithine, formaldehyde and acetone dicarboxylic acid. It is now considered[19] that the pyridine ring is derived from lysine. A modified Robinson's scheme has served as a working hypothesis in the present study and is shown in Fig. 1. It is postulated that α-keto-ϵ-aminocaproic acid (I) (derived from lysine by oxidative deamination) undergoes an enzyme-catalyzed Mannich type reaction with 4-aminobutanal (II) (from ornithine) leading to compound III. Decarboxylation and loss of water yields the tetrahydronornicotine (IV) which on dehydrogenation and N-methylation gives nicotine (V). The location[16,17] of the radioactivity in the nicotine obtained after feeding ornithine-2-C^{14} is consistent with this hypothesis and will be discussed together with the results obtained in the present work.

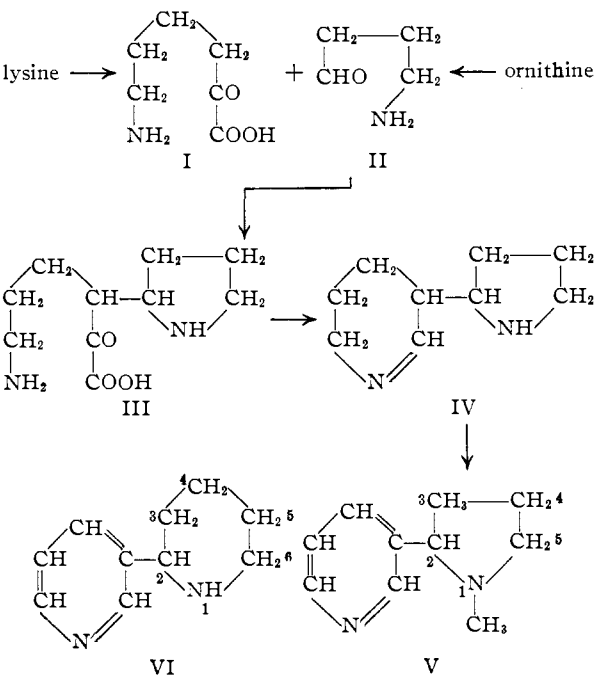

Fig. 1.

(1) Presented in part at the 128th meeting of the American Chemical Society, Minneapolis, Minn., Sept., 1955.

(2) Earlier work on nicotine biogenesis has been reviewed by (a) R. F. Dawson, *Advances in Enzymol.*, **8**, 217 (1948) and by (b) W. O. James in "The Alkaloids," edit. by R. H. F. Manske and H. L. Holmes, Vol. I, Academic Press, New York, N. Y., 1950, p. 60.

(3) S. A. Brown and R. U. Byerrum, THIS JOURNAL, **74**, 1523 (1952).

(4) L. J. Dewey, R. U. Byerrum and C. D. Ball, *ibid.*, **76**, 3997 (1954).

(5) R. U. Byerrum and R. E. Wing, *J. Biol. Chem.*, **205**, 637 (1953).

(6) R. U. Byerrum, R. L. Hamill and C. D. Ball, *ibid.*, **210**, 645 (1954).

(7) R. U. Byerrum, R. L. Ringler and R. L. Hamill, *Federation Proc.*, **14**, 188 (1955).

(8) R. U. Byerrum, J. D. Lovell and C. D. Ball, *Plant Physiol.*, Proc. of the Plant Physiology Meeting, East Lansing, Mich., Sept. 4–8, 1955, p. XVI.

(9) A. M. Kuzin and V. I. Merenova, *Doklady Akad. Nauk. S.S.S.R.*, **85**, 393 (1952).

(10) R. U. Byerrum and H. W. Culp, Abstracts of the 126th meeting of the American Chemical Society, Sept. 12–17, 1954, p. 16C.

(11) G. Klein and H. Linser, *Planta*, **20**, 470 (1933).

(12) R. F. Dawson, *Plant Physiol.*, **14**, 479 (1939).

(13) R. F. Dawson, D. R. Christman and R. C. Anderson, THIS JOURNAL, **75**, 5114 (1953).

(14) P. I. Mortimer, *Nature*, **172**, 74 (1953).

(15) K. Bowden, *ibid.*, **172**, 768 (1953).

(16) E. Leete, *Chemistry and Industry*, 537 (1955).

(17) L. J. Dewey, R. U. Byerrum and C. D. Ball, *Biochim. Biophys. Acta*, **18**, 141 (1955).

(18) R. Robinson, *J. Chem. Soc.*, **111**, 876 (1917).

(19) *Cf.* R. Robinson in "The Structural Relations of Natural Products," Oxford Univ. Press, London, 1955, pp. 67–70.

The biogenesis of anabasine (VI) in *N. glauca* has been discussed by Dawson[20] and this alkaloid seems to be synthesized in both the roots and the shoots of the intact plant. A biogenetic scheme analogous to that postulated for nicotine would depict its formation from two molecules of lysine. The *in vitro* synthesis of tetrahydroanabasine by dimerization of Δ^1-piperideine[21] (the aldimine from 5-aminopentanal) is strongly suggestive of such a biogenetic route.

In order to test this hypothesis, lysine-2-C^{14} was administered to intact *N. tabacum* and *glauca* by addition to the nutrient solution in which the roots of the plants were growing.

Experimental[22]

Cultivation of the Tobacco Plants.—The *N. tabacum* L. and *glauca* were grown from seed in soil. When the plants were about 5 months old they were transferred to a nutrient solution. This solution contained (per l. of distilled water) KNO_3 1.00 g., $Ca(NO_3)_2 \cdot 4H_2O$ 2.36 g., $MgSO_4 \cdot 7H_2O$ 1.00 g., KH_2PO_4 0.54 g., $FeSO_4 \cdot 7H_2O$ 4.0 mg., H_3BO_3 2.9 mg., $MnCl_2 \cdot 4H_2O$ 1.8 mg., $ZnSO_4 \cdot 7H_2O$ 0.22 mg., $CuSO_4 \cdot 5H_2O$ 0.08 mg., MoO_3 0.07 mg. The roots of each plant were suspended in about 700 ml. of this solution which was aerated continually and was changed once a week. The plants were grown under tubular fluorescent lights which were left on for about 16 hours of each day. The plants remained healthy and many new roots were produced.

Administration of the Lysine-2-C^{14} to the *N. tabacum* and Isolation of the Nicotine.—Lysine-2-C^{14} monohydrochloride[23] (627 mg.) with an activity of 1.03×10^7 c.p.m./m*M*[24] (total activity, 3.52×10^7 c.p.m.) was divided equally between the nutrient solutions of three 6 month old plants. The activity of the solutions decreased and after 7 days they contained only 0.7% of the original activity. After 8 days in contact with the radioactive lysine the plants were harvested and partially dried at 50°. The roots showed the highest activity with only low activity in the leaves. The nicotine was isolated from the plants by a modification of the method of Smith.[25] The plants were cut into small pieces and boiled with 1 l. of 0.1 *N* hydrochloric acid for 3 hours and then filtered. The residue was boiled with a further quantity of the dilute acid. The total activity of the combined extracts was 3.39×10^6 c.p.m. This solution was evaporated to small bulk, made alkaline with sodium hydroxide and extracted with ether. The ether extract was extracted three times with 100 ml. of 2 *N* hydrochloric acid, this was made alkaline and again extracted with ether. This final ether extract was dried over sodium sulfate and the ether evaporated to yield crude nicotine, 170 mg. This was distilled (120° (0.5 mm.)) and the distillate (124 mg.) was dissolved in 5 ml. of ethanol, cooled and 0.25 ml. of 70% perchloric acid added. Nicotine diperchlorate separated as colorless plates (170 mg.), m.p. 208–209°.

Anal. Calcd. for $C_{10}H_{14}N_2 \cdot 2HClO_4$: C, 33.07; H, 4.44. Found: C, 32.79; H, 4.54.

The nicotine diperchlorate was completely inactive.

Administration of the Lysine-2-C^{14} to *N. glauca* and Isolation of the Anabasine.—Lysine-2-C^{14} monohydrochloride (275 mg.) with an activity of 3.80×10^7 c.p.m./m*M* (total activity, 5.73×10^7 c.p.m.) was divided equally between the nutrient solutions of eight 6 month old plants. After 4 days no activity could be detected in the nutrient solutions. The plants were harvested after 16 days; at this time there was considerable activity in the leaves. The anabasine was isolated by a modification of the method of Feinstein, *et al.*[26] The fresh plant was macerated and mixed with 1500 ml. of chloroform and 150 ml. of 15 *N* ammonia solution and allowed to stand at room temperature with occasional shaking for 48 hours. The mixture was then filtered and the residue washed with more chloroform. The total chloroform layer was extracted four times with 100 ml. of 2 *N* sulfuric acid. The acidic layer was made alkaline with ammonia and extracted with chloroform, the extract being finally dried over sodium sulfate. The chloroform was removed *in vacuo* to leave the crude alkaloid which had a total activity of 3.12×10^4 c.p.m. A sample of this was chromatographed on paper buffered[27] to *p*H 7. The developing solvent was a mixture of 800 ml. of butanol and 150 ml. of water. The alkaloids were detected with Dragendorff's reagent.[28] With this system anabasine had an R_F value of 0.71 and nicotine 0.97. Only anabasine was detected in the extract of the *N. glauca*. The paper was subjected to radioactivity assay and the bulk of the activity was coincident with the position of the anabasine on the paper. The crude anabasine was distilled *in vacuo* (110–120° (0.1 mm.)) to yield 480 mg. of a pale yellow oil ($n^{25}D$ 1.5426) which solidified on cooling at 0° melting again at about 20°.

Anal. Calcd. for $C_{10}H_{14}N_2$: C, 74.03; H, 8.70. Found: C, 74.03; H, 8.47.

The anabasine (450 mg.) was dissolved in 2 ml. of ethanol, cooled and 0.9 ml. of 70% perchloric acid added. Excess ethyl ether was added and anabasine diperchlorate separated as an amorphous white powder (0.94 g.), m.p. 154–155°.

Anal. Calcd. for $C_{10}H_{14}N_2 \cdot 2HClO_4$: C, 33.07; H, 4.44. Found: C, 32.87; H, 4.68.

Anabasine dipicrate was prepared from the perchlorate by dissolving it in ethanol and adding an ethanolic solution of picric acid; the dipicrolonate was prepared similarly.

Degradation of the Anabasine.—The active anabasine diperchlorate (500 mg.) was dissolved in 10 ml. of concentrated nitric acid, one drop of ethanol was added and the mixture was heated on a steam-bath for 18 hours. Copious brown fumes were evolved. The solution was evaporated to dryness *in vacuo*, excess ammonia added and evaporated again. The residue was dissolved in water and a hot solution of cupric acetate added, the blue precipitate of the copper salt of nicotinic acid which separated out was filtered off and washed with water. The copper salt was decomposed by passing hydrogen sulfide into a suspension of it in hot water. Evaporation of the filtrate yielded a pale brown solid which was crystallized from ethanol and then sublimed (150° (0.01 mm.)) to yield nicotinic acid (52 mg.), m.p. 232° not depressed on admixture with an authentic specimen. The hydrochloride (m.p. 272°) and nitrate (m.p. 194°) were obtained by addition of the appropriate acid to ethanolic solutions of the nicotinic acid.

The nicotinic acid (13.3 mg.) was decarboxylated by refluxing in 10 ml. of quinoline with 20 mg. of copper chromite catalyst[29] at 280° in a nitrogen atmosphere for 3 hours. The carbon dioxide evolved was passed into a 5% solution of barium hydroxide. The barium carbonate was filtered off, washed with water, ethanol and ether; yield 15.5 mg.

The activities of anabasine and its degradation products are shown in Table I.

In a preliminary feeding experiment the same amount of lysine-2-C^{14} with the same activity was fed to eight *N. glauca* plants which were harvested after 5 days in contact with the radioactive amino acid. The anabasine was isolated and converted to the diperchlorate which had an activity of 7.7×10^2 c.p.m./m*M*. This was too low for the results from a degradation to be significant so the feeding was repeated, allowing the lysine to be metabolised in the plant for 16 days as described.

(20) Ref. 2a, pp. 223, 227.

(21) C. Schöpf, A. Komzak, F. Braun and E. Jacobi, *Ann.*, **559**, 1 (1948).

(22) All melting points are corrected, analyses were performed by Miss Heather King of this department.

(23) The lysine-2-C^{14} was prepared from C^{14} methyl labeled sodium acetate (purchased from Isotope Specialties, Glendale, Calif.) by established methods: E. Leete and L. Marion, *Can. J. Chem.*, **31**, 126 (1953); M. Fields, D. E. Walz and S. Rothchild, THIS JOURNAL, **73**, 1000 (1951).

(24) All counts were carried out in a windowless flow G. M. counter (Nuclear Instrument and Chemical Co. Model D-46 A) using "Q gas" as the quenching gas. Determinations were carried out on the actual organic compounds making corrections for self absorption. The preparation of the sample was standardized and results reproducible to 5% were obtained.

(25) H. H. Smith and C. R. Smith, *J. Agr. Research*, **65**, 347 (1942).

(26) L. Feinstein, P. J. Hannan and E. T. McCabe, *Ind. Eng. Chem.*, **43**, 1402 (1951).

(27) E. Leete, S. Kirkwood and L. Marion, *Can. J. Chem.*, **30**, 749 (1952).

(28) R. Munier and M. Macheboeuf, *Bull. soc. chim. biol.*, **33**, 849 (1951).

(29) W. A. Lazier and H. R. Arnold, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., p. 142.

TABLE I

	Specific activity (c.p.m./mM)
Anabasine diperchlorate	8.9×10^3
Anabasine dipicrate	9.2×10^3
Anabasine dipicrolonate	8.9×10^3
Nicotinic acid	8.8×10^3
Nicotinic acid hydrochloride	7.9×10^3
Nicotinic acid nitrate	8.7×10^3
Barium carbonate	8.3×10^3

Discussion

The results indicate that lysine is not a precursor of the pyridine ring of nicotine or anabasine, at least not in the *Nicotiana* species that were studied. The specific activity of anabasine and its degradation products shown in Table I indicate that all the radioactivity in the anabasine is located on carbon 2(VI) of the piperidine ring. The complete absence of any randomization is strongly in support of lysine being a direct precursor of the piperidine ring of anabasine. It is of interest to compare this result with that obtained on feeding ornithine-2-C^{14} to the nicotine producing *N. tabacum*[16] and *rustica*.[17] In these feeding experiments only half the activity in the radioactive nicotine was located on carbon 2 of the pyrrolidine ring of nicotine (V). This is explainable if the 4-aminobutanal (II) arises from ornithine-2-C^{14} (VII) by a metabolic pathway shown in Fig. 2. The conversion of ornithine to glutamic-γ-aldehyde (VIII) has been shown to occur in *Neurospora crassa*[30] and in a rat liver preparation.[31] Work on the formation of proline

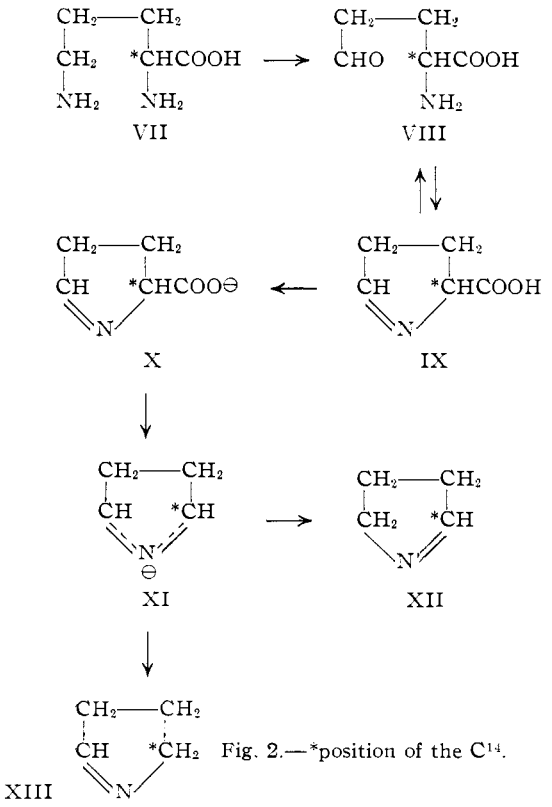

Fig. 2.—*position of the C^{14}.

from ornithine[32-34] also supports this reaction. Δ^1-Pyrroline-5-carboxylic acid (IX) is the aldimine in equilibrium with VIII. Decarboxylation of its carboxylate anion X would give the mesomeric anion XI which on protonation gives equal amounts of Δ^1-pyrroline-2-C^{14} (XII) and Δ^1-pyrroline-5-C^{14} (XIII). These are the ring closed aldimines from 4-amino-butanal-1-C^{14} and 4-amino-butanal-4-C^{14} respectively; subsequent Mannich reaction of this mixture as in Fig. 1 would give nicotine equally labeled on the 2- and 5-positions with C^{14}.

Since all the activity in the anabasine is located on C-2, the biosynthesis of its piperidine ring from lysine is obviously different from the biosynthesis of the pyrrolidine ring of nicotine from ornithine. In the formation of pipecolic acid (piperidine-2-carboxylic acid) from lysine,[35] the intermediate product seems to be α-keto-ϵ-aminocaproic acid (I)[36,37] which is in equilibrium with Δ^1-piperideine-2-carboxylic acid. Since the decarboxylation of this acid would lead to the higher homolog of the mesomeric ion XI and consequent randomization of activity between the 2- and 6-positions, it is suggested that decarboxylation occurs after the formation of a bond between the incipient pyridine ring and the 2-position of the Δ^1-piperidine-2-carboxylic acid.

Explanation of the failure of lysine to serve as a precursor of the pyridine ring of nicotine (and anabasine) may lie in the final step of the biogenetic scheme in Fig. 1. This is a dehydrogenation involving the removal of four hydrogen atoms and the plant enzyme system may not be able to carry out this reaction since this requires considerable energy, especially the conversion of the tetrahydro to a dihydropyridine. 3- or 4-hydroxylysine[38] may be more likely precursors of the pyridine ring. Thus replacing the lysine in Fig. 1 by 4-hydroxylysine and following through the biogenetic route to IV a 5-hydroxytetrahydropyridine derivative would be obtained. This will yield a dihydropyridine on dehydration, a reaction that requires less energy than dehydrogenation. The final step in the biogenesis of the pyridine ring will still be a dehydrogenation but this will be facilitated by the high resonance energy of the pyridine ring. The metabolism of radioactive 4-hydroxylysine in *Nicotiana* species is being examined.

It has been shown[39] that the feeding of uniformly labeled C^{14}-lysine to excised roots of *N. tabacum* growing in sterile culture yielded inactive nicotine. The administration of lysine labeled with N^{15} in the ϵ-position to the same culture yielded nicotine which contained a negligible amount of N^{15} in the pyridine ring and a low level of incorporation into the nitrogen of the pyrrolidine

(30) J. R. S. Fincham, *Biochem. J.*, **53**, 313 (1953).
(31) A. Meister, *J. Biol. Chem.*, **206**, 587 (1954).
(32) D. Shemin and D. Rittenberg, *ibid.*, **158**, 71 (1945).
(33) M. R. Stetten, *ibid.*, **189**, 499 (1951).
(34) T. Yura and H. J. Vogel, *Biochim. Biophys. Acta*, **17**, 582 (1955).
(35) P. H. Lowy, *Arch. Biochem. Biophys.*, **47**, 228 (1953).
(36) M. Rothstein and L. L. Miller, THIS JOURNAL, **76**, 1459 (1954).
(37) R. S. Schweet, J. T. Holden and P. H. Lowy, *J. Biol. Chem.*, **211**, 517 (1954); *Federation Proc.*, **13**, 293 (1954).
(38) A. I. Virtanen and S. Kari, *Acta Chem. Scand.*, **9**, 170 (1955), have isolated 4- and 5-hydroxy-2-pipecolic acid from the plant *Acacia pentadena* and these could have been produced from the 3- and 4-hydroxylysine, respectively.
(39) R. F. Dawson, A. Bothner By and D. R. Christman (private communication).

ring.[40] These results confirm those which are reported in this paper and substantiate our view that lysine is not a direct precursor of the pyridine ring of nicotine.

(40) R. F. Dawson and A. Bothner-By, private communication.

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LOS ANGELES 24, CALIFORNIA

[CONTRIBUTION FROM THE INSTITUTE OF HUMAN PHYSIOLOGY, UNIVERSITY OF MODENA]

Non-enzymatic Transamination between Peptides and Pyridoxal. Isolation of the 2,4-Dinitrophenylhydrazones of Some Ketopeptides

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Non-enzymatic transamination between pyridoxal and the peptides alanylglycine, leucylglycine, α- and γ-glutamylglycine has been compared with the same reaction for the corresponding N-terminal amino acids. The *p*H optimum of the reaction for the peptides as well as for the amino acids is about 4.5. Peptides react more slowly than amino acids. The 2,4-dinitrophenylhydrazones of the following ketopeptides have been isolated: pyruvoylglycine, pyruvoylalanine, α-ketoisocaproylglycine, α-ketoisocaproylglycylglycine, α-ketoisocaproyltyrosine and α-keto-α-glutarylglycine. Absorption spectra and chromatographic behavior of these compounds have been compared with those of the 2,4-dinitrophenylhydrazones of the corresponding keto acids.

It has been observed[1,2] that some peptides, when heated with pyridoxal in the presence of an aluminum salt as catalyst, undergo a non-enzymatic transamination reaction similar to that observed by Metzler and Snell[3] for the amino acids. Some features of this reaction are described in the present paper for a number of alanyl-, leucyl- and glutamylpeptides; the isolation of the 2,4-dinitrophenylhydrazones of the ketopeptides thereby formed is also reported.

Herbst and Shemin[4] demonstrated the formation of alanylalanine by non-enzymatic transamination between α-aminophenylacetic acid and pyruvoylalanine. The system employed here, in which transamination occurs between peptides and pyridoxal, is particularly interesting since pyridoxal phosphate is the coenzyme of transaminases. Snell, *et al.*,[5] have used this non-enzymatic system to study a whole series of amino acid reactions, which are catalyzed in the biological systems by pyridoxal phosphate-containing enzymes, and have found a perfect correspondence between the two reaction types.

The study of such a non-enzymatic transamination involving peptides can thence be considered of value for a re-examination of the possibility of the corresponding enzymatic reaction,[6] which some authors have credited with a possible role in peptide and protein synthesis.[7]

Experimental

Chemicals.—Pyridoxal was prepared from the ethylacetal hydrochloride (Hoffmann–LaRoche) according to Harris, *et al.*[8] Sodium pyruvate, sodium α-ketoglutarate, pyridoxamine dihydrochloride, DL-alanine (A), DL-alanylglycine (AG), L-leucine (L), L-leucylglycine (LG), L-leucylglycylglycine (LGG), L-leucyl-L-tyrosine (LT), L-glutamic acid (Glu) were also supplied by Hoffmann–La Roche. DL-Alanyl-DL-alanine (AA) was obtained from Light & Co.

Pyruvoylglycine (PG) was synthesized according to Bergmann and Grafe,[9] m.p. 89° (lit. value 90°).

α-L-Glutamylglycine (α-GluG) and γ-L-glutamylglycine (γ-GluG) were synthesized by the method of Sachs and Brand,[10] who found it suitable for the synthesis of other α- and γ-glutamylpeptides. Intermediate compounds were: N-carbobenzyloxy-L-glutamic acid γ-benzyl ester,[11] N-carbobenzyloxy-L-glutamic acid α-benzyl ester[12] and glycine benzyl ester hydrochloride.[13] The purity of the peptides was established by the analytical procedures recommended by Sachs and Brand.[10]

Anal. α-GluG. Calcd. for $C_7H_{12}O_5N_2$ (204.2): N, 13.7; amino N, 6.9; carboxyl N, 0.0. Found: N, 13.4; amino[14] N, 6.9; carboxyl[15]N, 0.1. γ-GluG. Calcd.: N, 13.7; amino-N, 6.9; carboxyl N, 6.9. Found: N, 13.4; amino[14]N, 13.2; carboxyl[15]N, 6.6.

(1) C. Cennamo, *Naturwissenschaften*, **41**, 39 (1954).

(2) C. Cennamo, *Ricerca sci.*, **25** (Suppl.: Giornate Biochimiche Italo-Franco-Elvetiche, Napoli, 21–24 Aprile, 1954), 331 (1955).

(3) D. E. Metzler and E. E. Snell, THIS JOURNAL, **74**, 979 (1952).

(4) R. M. Herbst and D. Shemin, *J. Biol. Chem.*, **147**, 541 (1943).

(5) D. E. Metzler, M. Ikawa and E. E. Snell, THIS JOURNAL, **76**, 648 (1954).

(6) Such a re-examination has been suggested lately once more by some authors (P. S. Cammarata and P. P. Cohen, *J. Biol. Chem.*, **187**, 439 (1950); A. Meister, *Advances in Enzymol.*, **16**, 185 (1955)). It is known, that several attempts to demonstrate a biological transamination for peptides have given negative results (A. E. Braunstein, *Enzymologia*, **7**, 25 (1939); P. P. Cohen, *J. Biol. Chem.*, **136**, 565 (1940)). The report of G. Ågren (*Acta Physiol. Scand.*, **1**, 233 (1940)) that glycyl-*p*-aminobenzoic acid and valylglycine can transaminate with α-ketoglutaric acid in the presence of minced cattle diaphragm muscle has been criticized (P. P. Cohen in J. B. Sumner and K. Myrbäck, "The Enzymes," Vol. 1, Part 2, Academic Press, Inc., New York, N. Y., 1951, p. 1040). More recent research in the field of enzymatic transamination, however, has shown that compounds, formerly believed incapable to react, do actually do so. Of particular interest here is the work of A. Meister (*Advances in Enzymol.*, **16**, 185 (1955)), who showed that biological transamination does occur for glutamine and asparagine, in contrast with previous results (A. Virtanen and T. Laine, *Biochem. Z.*, **308**, 213 (1941)). Non-enzymatic transamination has also been demonstrated for the same compounds (H. I. Nakada and S. Weinhouse, *J. Biol. Chem.*, **204**, 831 (1953); A. Meister, *ibid.*, **200**, 571 (1953); A. Meister and P. E. Fraser, *ibid.*, **210**, 37 (1954)).

(7) K. Linderstrøm-Lang, *Ann. Rev. Biochem.*, **8**, 49 (1939); R. Schönheimer, S. Ratner and D. Rittenberg, *J. Biol. Chem.*, **130**, 703 (1939).

(8) S. A. Harris, D. Heyl and K. Folkers, THIS JOURNAL, **66**, 2088 (1944).

(9) M. Bergmann and K. Grafe, *Hoppe-Seyler's Z. physiol. Chem.*, **187**, 187, 196 (1930).

(10) H. Sachs and E. Brand, THIS JOURNAL, **75**, 4608 (1953).

(11) W. E. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.*, 3239 (1950).

(12) H. Sachs and E. Brand, THIS JOURNAL, **75**, 4610 (1953).

(13) B. F. Erlanger and E. Brand, *ibid.*, **73**, 3508 (1951).

(14) Reaction time with nitrous acid was 3 minutes; with the γ-peptides both amino and peptide nitrogens react[10] (Van Slyke, manometric amino N procedure).

(15) D. D. Van Slyke, R. T. Dillon, D. A. MacFadyen and P. Hamilton, *J. Biol. Chem.*, **141**, 627 (1941). Reaction time with ninhydrin was 7 minutes at *p*H 2.5.