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.

Acknowledgment.—The author is indebted to Dr. S. G. Wildman and Dr. A. Lang of the Department of Botany of this University for help in the cultivation of the tobacco plants.

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

BY CARLO CENNAMO, BIANCAROSA CARAFOLI AND ERIK P. BONETTI

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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. I, 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.

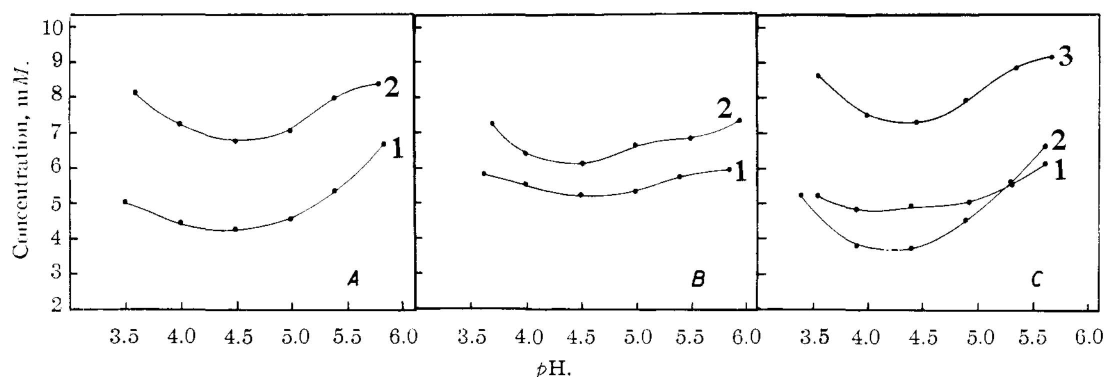


Fig. 1.—Pyridoxal concentration, after reaction with amino acids and peptides for 30 min. at 100° *versus* *p*H; initial concentration, 0.01 *M*; Graph A: 1, with leucine; 2, with leucylglycine. Graph B: 1, with alanine; 2, with alanylglycine. Graph C: 1, with glutamic acid; 2, with γ-glutamylglycine; 3, with α-glutamylglycine.

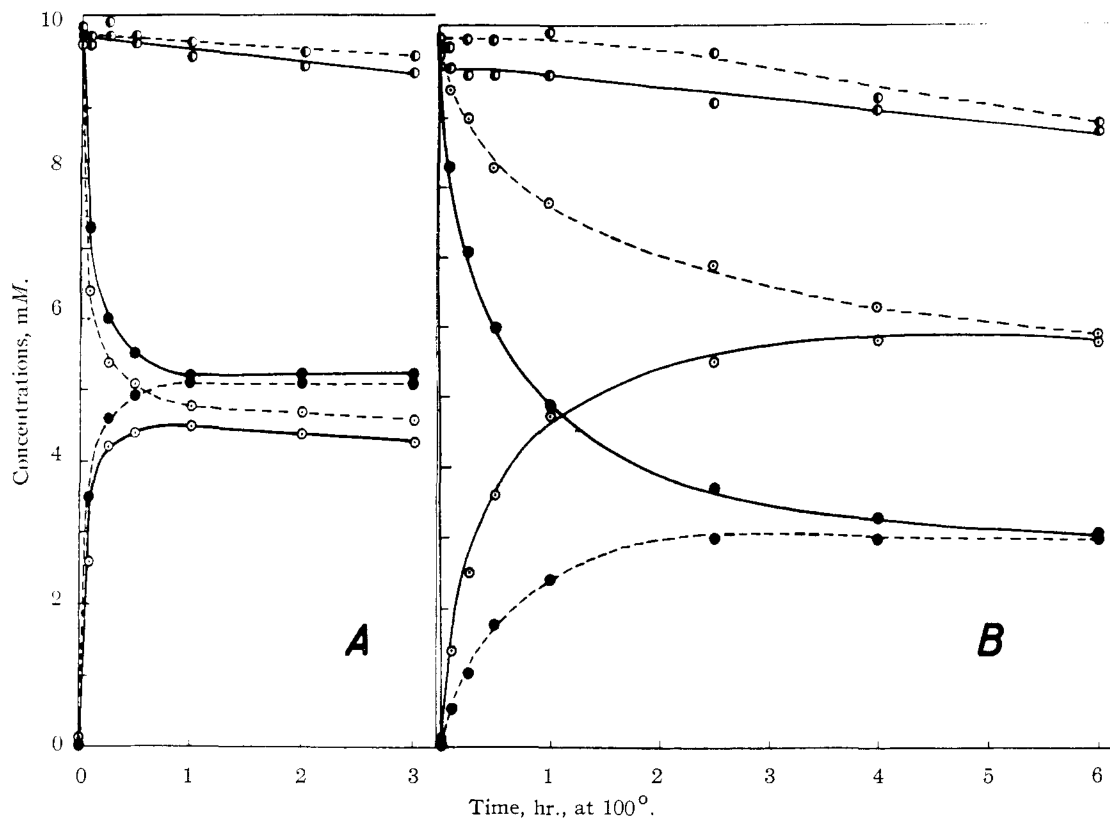


Fig. 2.—Pyridoxal and pyruvic acid (graph B) or pyruvoylglycine (graph A) concentrations *versus* time, during transamination at 100° and *p*H 5.0: ●, pyridoxal; ⊙, carbonyl compounds; ◐, sum of pyridoxal and carbonyl compounds. Graph B: reaction between pyridoxal and alanine (solid); reaction between pyridoxamine and pyruvic acid (dash). Graph A: reaction between pyridoxal and alanylglycine (solid); reaction between pyridoxamine and pyruvoylglycine (dash).

α-GluG and γ-GluG were readily separable by paper chromatography (see below), the R_f values being 0.17 and 0.13, respectively.

Stock solutions of the above compounds were made in redistilled water, stored in a refrigerator and used within a few days.

Reaction of Peptides with Pyridoxal.—The reaction mixtures were similar to those used by Metzler and Snell.[3] These mixtures contained 0.01 *M* pyridoxal (or pyridoxamine), 0.01 *M* substrate (amino acid, peptide, pyruvic acid or PG), 0.001 *M* $KAl(SO_4)_2 \cdot 12H_2O$ and 0.2 *M* acetate buffer, *p*H 5.0; total volume, 1.5 ml. Acetate buffers at varying *p*H were used to study the influence of *p*H on the reaction rate; *p*H measurements were made with a Beckman model G meter at room temperature.

The mixtures were first heated in sealed glass tubes in a boiling water-bath for the desired time, and then quickly chilled and appropriately diluted for the successive analyses.

Measurements of pyridoxal, total vitamin B_6 (pyridoxal + pyridoxamine) and pyruvic acid concentrations were made according to Metzler and Snell.[3] Pyridoxal determinations were made with a Beckman model DU spectrophotometer. To 2-ml. samples of the hundred-fold diluted mixtures was added 2 ml. of 50% ethanolamine and absorption was measured at 360 mμ, using one cm. cells. A linear calibration curve was obtained with 0.02–0.2 μ moles of pyridoxal per sample. The procedure employed for pyruvic acid was also used for the determination of PG; 3-ml. samples of the sixty-fold diluted mixtures were used (K = μ moles of PG per sample/photometric density = 0.75; Evelyn photoelectric colorimeter, filter no. 520).

With all the peptides used, transamination with pyridoxal

was negligible in the absence of alum. The same holds true for the reaction between PG and pyridoxamine.

Stability of the Reactants.—Mixtures with either no pyridoxal or no alum were employed to check the stability of peptides and of PG against hydrolysis, under our experimental conditions. This control was performed by paper chromatography, employing the descending method (paper, Whatman No. 1) and the upper layer of the system *n*-butanol (40)–acetic acid (10)–water (50) as solvent. Developed chromatograms were sprayed with ninhydrin. The different chromatographic behaviors of the peptides and of at least one of their constituent amino acids made the control possible in every case.

Alanyl- and leucyl-peptides,[16] as well as PG, appeared to be stable against prolonged heating (up to 6 hours). α-GluG underwent a slow hydrolysis after 4 hours of heating, whereas for γ-GluG hydrolysis became evident already after half an hour.

Paper chromatography was also used to follow pyridoxamine formation, during transamination reactions, as well as AG appearance, in transamination between pyridoxamine and PG.

Determinations of pyridoxal + pyridoxamine showed that during the transamination reactions these compounds were stable. A considerable decrease in total vitamin B_6 was observed in the transamination of α-GluG only, as shown below.

When heated alone for 6 hours PG showed a 5% decrease in color with the determination method used.

Isolation of the Ketopeptide 2,4-Dinitrophenylhydrazones.—Larger amounts (25 ml.) of the mixtures described above, now containing a tenfold concentration of amino acid or peptide (0.1 *M*) were heated at 100°, for 1 and 4 hours, respectively. Pyridoxal disappearance was always higher than 90%. The mixtures were chilled and acidified with concd. HCl; an excess of 2,4-dinitrophenylhydrazine (1% in 2 *N* HCl) was then added. Keto acid and ketopeptide phenylhydrazones (KAP's and KPP's), which precipitated immediately or on standing overnight in a refrigerator, were collected and dissolved, without heating, in the least amount of ethanol; a scarcely soluble residue of pyridoxal phenylhydrazone was filtered off. Crystallization occurred upon addition of water, and was repeated twice. In some cases (pyruvic acid and α-ketoisocaproic acid phenylhydrazones) a recrystallization from 10% Na_2CO_3–concd. HCl was found useful.

KPP isolation has been successful with all peptides used except γ-GluG. In this case no precipitation occurred on addition of 2,4-dinitrophenylhydrazine to the reaction product.[17] For comparative purposes, 2,4-dinitrophenylhydrazones were prepared from commercial sodium pyruvate and sodium α-ketoglutarate, and from synthetic PG. The α-ketoisocaproic acid phenylhydrazone was a gift of Prof. D. Cavallini.

Absorption spectra of the phenylhydrazones (3×10^{-5} *M* in 1.25 *N* NaOH) were measured, in the range of 400 to 600 mμ, with a Beckman model DU spectrophotometer. Melting points were determined by the capillary method and with the Kofler microstage apparatus. Nitrogen determinations were made with micro-Kjeldahl.

The chromatographic behavior of KPP's and KAP's, dissolved in 1 *N* NH_3, has been examined. The paper was Schleicher and Schüll no. 2043 b; the upper layer of the mixture *n*-butanol (40)–ethanol (10)–water (50) was used as solvent.[18] The strips were previously equilibrated for 4 hours with the vapors of the solvent. A few drops of concd. NH_3 were let fall in the tank. Developed chromatograms were sprayed with *n*-butanol saturated with 2.5 *N* NaOH. The colors so obtained lasted for some hours.

pH Dependence of the Reaction.—Metzler and Snell[3] observed that the *p*H optimum of the reaction between Glu and pyridoxal is about 4.5.

The *p*H dependence of the transamination of AG, LG, α-GluG and γ-GluG was examined here in comparison with that of the corresponding N-terminal amino acids. In the graphs of Fig. 1, residual pyridoxal concentrations, after 30 minutes of heating at 100°, are plotted *versus* *p*H. In all instances, the *p*H optimum is near 4.5. *p*H values higher than 6.0 were not tested, since it was previously observed that, in alkaline medium and with some peptides (AG and LG), pyridoxal loss was not due to transamination only, but also to some other reaction, not requiring aluminum, that probably involves the formation of condensation compounds.[2]

Equilibrium Position and Reaction Rate.—The equilibrium positions reached in the transamina-

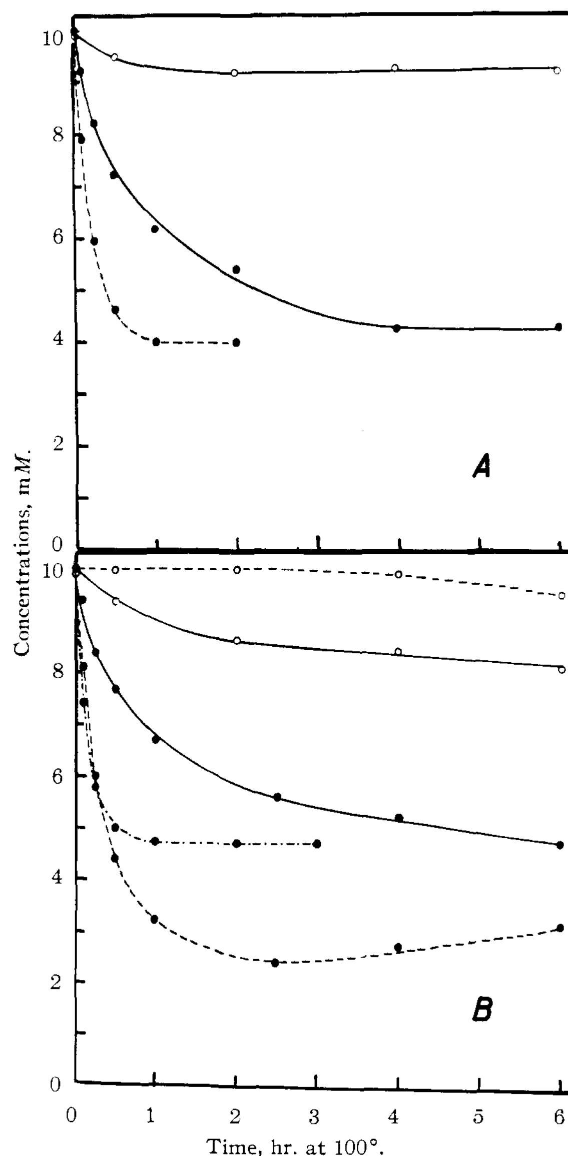


Fig. 3.—Pyridoxal and total vitamin B_6 concentrations *versus* time, during transamination at 100° and *p*H 5.0: ●, pyridoxal; O, total vitamin B_6. Graph A: reaction of pyridoxal with leucine (dash) and with leucylglycine (solid). Graph B: reaction of pyridoxal with glutamic acid (dash-dot), with α-glutamylglycine (solid) and with γ-glutamylglycine (dash).

(16) LT was previously[1] reported to undergo hydrolysis on heating with pyridoxal in the absence of alum; a spot, wrongly assigned to leucine, was indeed observed. However, this spot did not appear in the absence of pyridoxal and was likely due to a compound formed by a secondary reaction between pyridoxal and the tyrosine residue.

(17) An attempt to detect chromatographically a possible soluble phenylhydrazone gave negative results.

(18) D. Cavallini and N. Frontali, *Ricerca sci.*, **23**, 807 (1953).

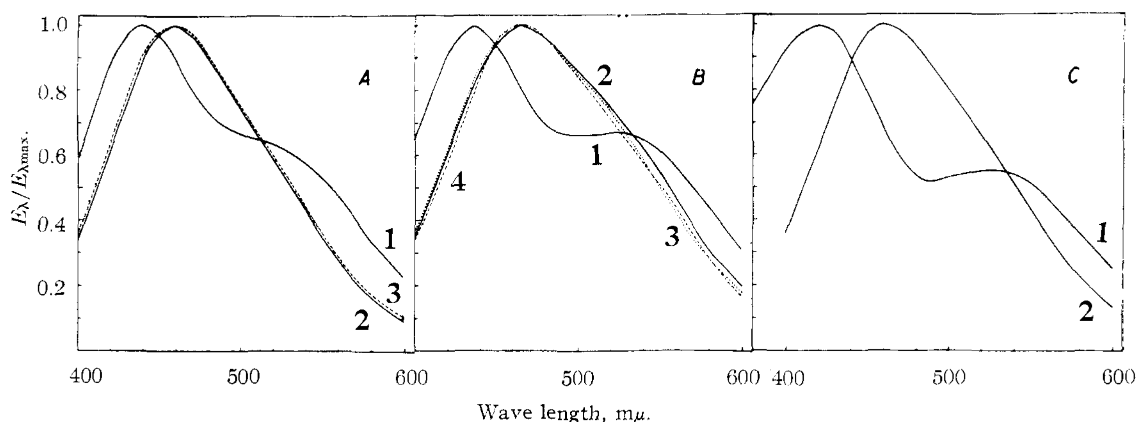


Fig. 4.—Relative absorption spectra of 2,4-dinitrophenylhydrazones, 3×10^{-5} *M* in 1.25 *N* NaOH: Graph A: 1, pyruvic acid; 2, pyruvoylglycine; 3, pyruvoylalanine. Graph B: 1, α-ketoisocaproic acid; 2, α-ketoisocaproylglycine; 3, α-ketoisocaproylglycylglycine; 4, α-ketoisocaproyltyrosine. Graph C: 1, α-ketoglutaric acid; 2, α-keto-α-glutarylglycine.

tion of the four above-mentioned peptides and of the corresponding N-terminal amino acids have been determined by following the drop in pyridoxal concentration with time, at 100° and *p*H 5.0. The values obtained for these amino acids agree with those given by Metzler and Snell.[3] In the case of A and AG (Fig. 2), also the reverse reactions, between pyruvate or PG and pyridoxamine have been followed, and carbonyl compounds have been measured also.

In the AG system, both forward and reverse reactions reach the same equilibrium position, and the sum of the reactant concentrations falls very slowly with time; a parallel chromatographic test shows progressive formation of AG on reaction of PG with pyridoxamine. AG reacts with pyridoxal more slowly than A, but the equilibrium position is clearly different and is attained when 30% (instead of 50%) of the vitamin B_6 is present as pyridoxal.

Figure 3 (graph A) shows the time course of the transamination for L and LG.LG reacts more slowly, but the same equilibrium position is reached as with L; at the equilibrium, 40% of the vitamin B_6 is present as pyridoxal, in both cases.

Figure 3 (graph B) likewise shows the time course of the transamination for Glu, α-GluG and γ-GluG. No comparison is possible in this case. α-GluG reacts more slowly than Glu; the considerable drop in pyridoxal + pyridoxamine concentration and the occurrence of some hydrolysis (see above) may alter the true equilibrium position. Apparently, transamination is faster for γ-GluG than for Glu, and the equilibrium position also appears to be quite different. However, the fact that α-keto-γ-glutarylglycine could not be isolated as phenylhydrazone suggests that, once formed, it undergoes a secondary reaction masking the ketonic group.[19] Moreover, some hydrolysis of γ-GluG takes place at the same time, as already mentioned above. Both facts lead to the conclusion that here the time course of the transamination is altered.

Ketopeptide 2,4-Dinitrophenylhydrazones.—The phenylhydrazones of the following ketopeptides were isolated after transamination of corresponding peptides with pyridoxal: pyruvoylglycine (PG), pyruvoylalanine (PA), α-ketoisocaproylglycine (KICG), α-ketoisocaproylglycylglycine (KICGG), α-ketoisocaproyltyrosine (KICT), α-keto-α-glutarylglycine (KGluG). Nitrogen content, melting points, R_f values and molar extinction coefficients at λ_{max} (3×10^{-5} *M* solutions in 1.25 *N* NaOH) of these KPP's are given in Table I. R_f values and ϵ values at λ_{max} for pyruvic acid (P), α-ketoisocaproic acid (KIC), and α-ketoglutaric acid (KGlu) phenylhydrazones are also reported for comparative purposes.

TABLE I

NITROGEN CONTENT, MELTING POINTS, MOLAR EXTINCTION COEFFICIENTS AND R_f VALUES OF 2,4-DINITROPHENYLHYDRAZONES ISOLATED AFTER TRANSAMINATION OF PEPTIDES WITH PYRIDOXAL (SEE TEXT)

2,4-Dinitrophenylhydrazone of	Mol. wt.	N, % Calcd.	N, % Found	M.p., °C.	$\epsilon \times 10^{-3}$	at mμ	R_f
P					19.6	440	0.51; 0.69
PG	325.2	12.9	13.2	245–246[a]	26.7[a]	460	0.45
PA	339.3	12.4	12.3	215–217	26.8	460	.59
KIC					17.7	435	0.75; 0.83
KICG	367.3	11.4	11.4	97–98; 161–162[b]	22.5	465	0.69
KICGG	424.4	13.2	12.9	157–159	22.8	465	.58
KICT	473.4	8.9	8.7	98–99[c]	23.1	465	.77
KGlu					15.4	420	.25
KGluG	383.3	11.0	10.7	115–116	24.2	460	.17

[a] The same value was obtained with the phenylhydrazone of the synthetic compound. [b] Resolidification occurs at about 135°. [c] No resolidification occurs.

In Fig. 4, relative spectrophotometric readings for isolated KAP's and KPP's (3×10^{-5} *M* in 1.25 *N* NaOH) are plotted against λ, allowing thereby a better comparison between KAP's and KPP's.

The ϵ values at λ_{max}, given in Table I, and the curves of Fig. 4 show that KPP's containing the

(19) A. Meister (*J. Biol. Chem.*, **200**, 571 (1953)) found that α-ketoglutaramic and α-ketosuccinamic acids (the α-keto analogs of glutamine and asparagine) can exist in two forms, the one exhibiting properties characteristic of a free α-keto group, thus readily forming a 2,4-dinitrophenylhydrazone, the other lacking to show such a behavior. This latter inactive form, which is suggested by Meister to possess a cyclic structure, and is particularly stable in the case of α-ketoglutaramic acid, is the only one that occurs in the case of α-keto-N-methylglutaramic acid. Considering the similarity existing between α-keto-N-methylglutaramic acid and α-keto-γ-glutarylglycine, it is easily conceivable that the latter compound also exists in a non-reactive cyclic form.

same ketoacid exhibit essentially the same absorption spectrum in the range of 400 to 600 mμ. Furthermore, while the spectra of the single KAP's differ widely, those of KPP's are all very similar. KPP spectra, if compared to corresponding KAP spectra, show higher values of λ_{max} and of ϵ at λ_{max} and are characterized by the absence of the plateau between 480 and 540 mμ.

By paper chromatography, all KPP's give single yellow spots, which turn reddish after spraying with *n*-butanol saturated with 2.5 *N* NaOH (KAP main spots turn brown; faster spots, when present, do not change their yellow color).

The availability of synthetic PG allowed a comparison of its phenylhydrazone with the one isolated after transamination between AG and pyridoxal. The two compounds exhibited identical melting points (no depression by mixing), absorption spectra and R_f values.

MODENA, ITALY

[CONTRIBUTION FROM THE DIVISION OF CANCER RESEARCH, DEPARTMENT OF SURGERY, UNIVERSITY OF ROCHESTER MEDICAL CENTER]

A Method for the Synthesis of C_{21}-Labeled Cholesterol

BY P. KURATH AND MARGARET CAPEZZUTO

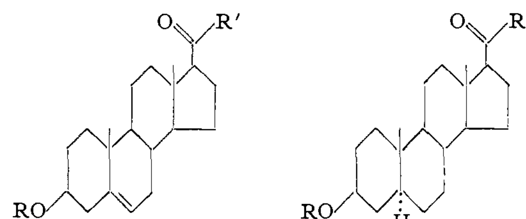
RECEIVED SEPTEMBER 29, 1955

A modification of Woodward's[3] and Robinson's[4] synthesis of the cholesterol side chain is described. This modification involves an alteration of the sequence of addition of carbon atoms to the C-20 acid. In this work carbon atom 21 is added after the addition of carbon atoms 22–27 to the C-20 acid. The synthesis was used to prepare cholestanol-21-^{14}C acetate which was then converted by known procedures to cholesterol-21-^{14}C.

The fact that cholesterol is a precursor of the adrenocortical hormones has been established.[1,2] What remains to be clarified, however, is the way in which the cholesterol side chain is degraded during the biosynthesis of the C-21 steroids. One approach to the study of the latter problem would be to utilize cholesterol labeled at C_{21}. Accordingly this report presents a method for the synthesis of such a labeled cholesterol.

Previous publications by Woodward[3] and Robinson[4] describing the total synthesis of cholesterol offered a method for the preparation of the C_{21}-labeled compound. For practical reasons it seemed more desirable to introduce the labeled carbon atom as late as possible. Therefore, the isohexyl side chain (carbon atoms 22–27) was added first to the C-20 acid followed by the introduction of the methyl group (carbon atom 21) as the last step to complete the carbon skeleton of cholesterol. A similar sequence of steps was followed in the syntheses of the 20α- and 20β-hydroxy compounds of the Δ^5-3β-hydroxy-24,24-dimethylcholen and of the corresponding saturated compound.[5]

As a convenient starting material for the synthesis, Δ^5-3β-acetoxyetiocholenic acid (I)[6] was chosen and converted into its acid chloride II.[5,7] The latter (II) was then allowed to react with diisohexylcadmium to yield 21-nor-20-ketocholesterol acetate (III).[8] Upon saponification of III, the free alcohol IV[8] was obtained. Hydrogenation of III yielded 21-nor-20-ketocholestanol acetate (VII).



I, R = Ac, R′ = OH V, R = Ac, R′ = OH
II, R = Ac, R′ = Cl VI, R = Ac, R′ = Cl
III, R = Ac, R′ = isohexyl VII, R = Ac, R′ = isohexyl
IV, R = H, R′ = isohexyl VIII, R = H, R′ = isohexyl

In another approach to the synthesis of VII, the acid acetate I was reduced to 3β-acetoxyetioallocholanic acid (V),[6] followed by the conversion to the corresponding acid chloride VI[3,5] which yielded with diisohexylcadmium the desired 21-nor-20-ketocholestanol acetate (VII). Upon mild saponification 21-nor-20-ketocholestanol (VIII) was obtained.

The acetate, VII, as well as the free alcohol, VIII, on reaction with methylmagnesium iodide yielded a mixture of the two possible stereoisomeric 20-hydroxycholestanols.[3,4] This mixture after treatment with acetic acid and acetic acid–acetic anhydride[3,4,9] yielded, upon hydrogenation over reduced platinum oxide,[3] cholestanol acetate (IX), which was identical with the natural product. Saponification of the acetate IX yielded cholestanol

(1) For reviews on the subject see, *e.g.*, O. Hechter in "Ciba Foundation Colloquia on Endocrinology, Volume VII, Synthesis and Metabolism of Adrenocortical Steroids," Little, Brown and Co., Boston, 1953, p. 161; S. Lieberman and S. Teich, *Pharmacol. Revs.*, **5**, 285 (1953).

(2) N. Saba, O. Hechter and D. Stone, THIS JOURNAL, **76**, 3862 (1954); W. S. Lynn, Jr., E. Staple and S. Gurin, *ibid.*, **76**, 4048 (1954); E. Reich and A. L. Lehninger, *Biochim. Biophys. Acta*, **17**, 136 (1955).

(3) R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler and W. M. McLamore, THIS JOURNAL, **74**, 4223 (1952); R. B. Woodward, F. Sondheimer and D. Taub, *ibid.*, **73**, 3548 (1951).

(4) H. M. E. Cardwell, J. W. Cornforth, S. R. Duff, H. Holtermann and R. Robinson, *J. Chem. Soc.*, 361 (1953).

(5) J. R. Billeter and K. Miescher, *Helv. Chim. Acta*, **32**, 564 (1949).

(6) M. Steiger and T. Reichstein, *ibid.*, **20**, 1040 (1937).

(7) M. Steiger and T. Reichstein, *ibid.*, **20**, 1164 (1937).

(8) A. Wettstein (*ibid.*, **23**, 1371 (1940)) obtained the free alcohol IV by treating the acid chloride II with the diethyl ester of the sodium derivative of isoamylmalonic acid followed by the saponification and the decarboxylation of the intermediate β-ketoester. Upon acetylation of IV the acetate III was obtained.

(9) *Cf.* A. Butenandt and H. Cobler, *Z. physiol. Chem.*, **234**, 218 (1935); A. Butenandt and G. Müller, *Ber.*, **71**, 191 (1938); B. Koechlin and T. Reichstein, *Helv. Chim. Acta*, **27**, 549 (1944).