$-36.5°$ (c 2% in methanol), was prepared as described previously.[15]

Acetyl-L-phenylalaninamide (II).—Esterification of 4.7 g. of acetyl-L-phenylalanine, prepared by the acetylation of L-phenylalanine,[16] with methanolic hydrogen chloride, gave 4 g. of acetyl-L-phenylalanine methyl ester, m.p. 89–90°, large prisms, after three recrystallizations from ether; $[\alpha]^{25}D$ $+19.5°$ (c 2% in methanol).

Anal. Calcd. for $C_{12}H_{15}O_3N$ (221): C, 65.2; H, 6.8; N, 6.3. Found: C, 65.0; H, 6.8; N, 6.0.

Ammonolysis of the above ester gave II, fine needles, m.p. 176–177°, after two recrystallizations from water, $[\alpha]^{25}D$ $+27°$ (c 1% in methanol).

Anal. Calcd. for $C_{11}H_{14}O_2N_2$ (206): C, 64.1; H, 6.8; N, 13.6. Found: C, 64.2; H, 6.8; N, 13.6.

Acetyl-D-phenylalanine Methyl Ester (IV).—To a solution of 22.1 g. of acetyl-DL-phenylalanine methyl ester in 75 ml. of methanol and 500 ml. of water, contained in a beaker thermostated at 30° was added 50 mg. of α-chymotrypsin and the pH of the solution maintained at approximately 7.8 by the addition of 1 N aqueous sodium hydroxide. Although the reaction appeared to be completed in two hours, the solution was stirred for another half-hour and then evaporated, at room temperature in a current of air, to about 200 ml. The reaction mixture was stored at 0° overnight, the precipitate recovered, washed with a small quantity of cold water and dried in air to give 9.7 g. of crude IV. The crude IV was recrystallized twice from ether to give IV, dense prisms, m.p. 90–91°, $[\alpha]^{25}D$ $-19°$ (c 2% in methanol).

Anal. Calcd. for $C_{12}H_{15}O_3N$ (221): C, 65.2; H, 6.8; N, 6.3. Found: C, 65.2; H, 6.8; N, 6.4.

Acetyl-D-phenylalaninamide (V).—Ammonolysis of 1 g. of IV gave 0.6 g. of V, fine needles, m.p. 176–177°, after two recrystallizations from water; $[\alpha]^{25}D$ -27 (c 1% in methanol).

Anal. Calcd. for $C_{11}H_{14}O_2N_2$ (206): C, 64.1; H, 6.9; N, 13.6. Found: C, 64.2; H, 6.8; N, 13.6.

Enzyme Experiments.—The methods used were identical with those reported previously.[3] The enzyme preparation, lot no. 90402, was obtained from Armour and Company. The K_S values for the two substrates employed in the inhibition experiments are nicotinyl-L-tryptophanamide, 2.7×10^{-3} M,[3] acetyl-L-tyrosinamide, 30.5×10^{-3} M.[4] It will be noted that the specific enzyme concentrations, E'_S and E'_I, for each series of experiments were such as to provide essentially zone A conditions for all experiments.[17,18] For both of the specific substrates, at the concentrations experimentally feasible, the course of hydrolysis approximated a first-order reaction, and initial velocities were calculated from the respective apparent first order rate constants.

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(16) H. T. Huang and C. Niemann, *ibid.*, **73**, 475 (1951).

(17) O. H. Straus and A. Goldstein, *J. Gen. Physiol.*, **26**, 559 (1943).

(18) A. Goldstein, *ibid.*, **27**, 529 (1944).

PASADENA, CALIFORNIA RECEIVED MAY 29, 1951

[CONTRIBUTION FROM THE U. S. PUBLIC HEALTH SERVICE, TUBERCULOSIS RESEARCH LABORATORY, CORNELL UNIVERSITY MEDICAL COLLEGE]

Glutamic γ-Semialdehyde and Δ^1-Pyrroline-5-carboxylic Acid, Intermediates in the Biosynthesis of Proline[1,2]

BY HENRY J. VOGEL[3] AND BERNARD D. DAVIS

Δ^1-Pyrroline-5-carboxylic acid (V) has been synthesized from γ,γ-dicarbethoxy·γ-acetamidobutyraldehyde with intermediate formation of glutamic γ-semialdehyde. The structure of V has been confirmed by its catalytic reduction to proline and its reaction with o-aminobenzaldehyde. V satisfies the proline requirement of one *Escherichia coli* mutant and appears to be structurally identical with a proline precursor (A) accumulated by another; a third mutant responds to proline, A or glutamic acid. The accumulation of A is enhanced by addition of its precursor, glutamic acid, or of o-aminobenzaldehyde, which functions as an effective "trapping agent." A microbiological assay method for proline or V has been described. The present results support the following scheme of proline biosynthesis: glutamic acid → glutamic γ-semialdehyde → Δ^1-pyrroline-5-carboxylic acid → proline.

The route of biosynthesis of proline in certain microörganisms has been reported to proceed *via* glutamic acid[4,5]; a relationship between the two amino acids has also been shown in mammalian metabolism.[6–14] The possibility of throwing additional light on proline biosynthesis arose when a proline-requiring mutant of *Escherichia coli* (55-1) was found to accumulate in its culture filtrate a substance (A), presumably a proline precursor, which supported growth of another proline auxotroph (55-25).[15] A third mutant (22-64) responded alternatively to proline, A or glutamic acid. These growth responses together with the accumulation of A indicate that glutamic acid, A, and proline form a biosynthetic sequence. The proline requirement of strain 22-64 is much larger than that of the other two strains and is of the order of magnitude of its glutamic acid requirement; it therefore appears that in strain 22-64 glutamic acid is formed from proline by a reversal of the above sequence.

The studies in mammals or with mammalian enzymes have primarily been concerned with the conversion of proline to glutamic acid; among the compounds that have been considered as possible intermediates in this process are α-amino-δ-hydroxyvaleric acid (I),[7] glutamic γ-semialdehyde (II)[11] and 2-pyrrolidone-5-carboxylic acid (III),[7] all of which may be regarded as derived from glutamic acid by some modification of its γ-carboxyl group. While in mammals I and III have been shown to be unlikely as intermediates,[7] compounds

(1) Aided by a grant from the Rockefeller Foundation.

(2) Preliminary note: H. J. Vogel and B. D. Davis, *Federation Proc.*, **10**, 423 (1951).

(3) U. S. Public Health Service Research Fellow.

(4) E. L. Tatum, *Proc. Natl. Acad. Sci. U. S.*, **31**, 215 (1945).

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(6) H. Weil-Malherbe and H. A. Krebs, *Biochem. J.*, **29**, 2077 (1935).

(7) M. Neber, *Z. physiol. Chem.*, **240**, 70 (1936).

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(12) J. V. Taggart and R. E. Krakaur, *ibid.*, **177**, 641 (1949).

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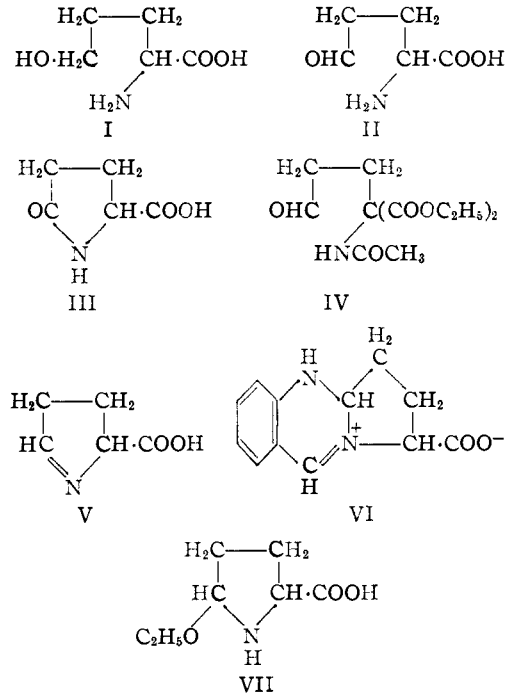
(14) M. R. Stetten, *J. Biol. Chem.*, **189**, 499 (1951).

(15) B. D. Davis, *Experientia*, **6**, 41 (1950).

at the aldehyde stage of oxidation of the γ-carboxyl carbon atom have been postulated repeatedly[10,11,13,14]; more direct evidence that II may be an oxidation product of proline has come from studies with the cyclophorase system.[12] In *Neurospora* I is utilized, although after an initial lag, by certain proline auxotrophs, and a hypothetical role of II has been considered[16]; in *Micrococcus pyogenes* III is able to substitute for glutamic acid, but not for proline, at the relatively high concentration of 1000 γ/ml.[17]

When tested with the three *E. coli* mutants described here, I and III were found to be inactive. Preliminary experiments undertaken to test the possibility of a biosynthetic intermediate between glutamic acid and proline at the aldehyde stage of oxidation gave positive results; careful oxidation of I yielded a product which, without isolation, had activity, like A, for strains 55-25 and 22-64, but not for 55-1.



Attempts to isolate A in a pure state from culture filtrates of strain 55-1 failed. While dilute solutions of A were relatively stable, biological activity was lost whenever concentration was attempted; this activity could be regenerated partly on heating in dilute aqueous solution. This behavior, suggestive of polymerization, would be consistent with formulation of A as II or a cyclized form thereof. Cyclization would be expected, since it has been shown that γ-aminoaldehydes and related compounds give intramolecular cyclization products with which under certain conditions they appear to be in equilibrium[18–20]; at physiological *p*H they have been reported to react in the cyclized form.[18,19] The possibility that in this class of compounds ring formation may be essentially complete is suggested by the fact that two substances presumed to be γ-aminoaldehydes did not react with an aldehyde oxidase.[12,21]

Accordingly, the synthesis of cyclized II, Δ^1-pyrroline-5-carboxylic acid (V), has now been undertaken. On refluxing 1 millimole γ,γ-dicarbethoxy-γ-acetamidobutyraldehyde (IV) in 6 *N* hydrochloric acid, a product was obtained which was very nearly as active as 1 millimole of DL-proline for strains 55-25 and 22-64. The product was inactive for strain 55-1 and hence contained no proline. At neutral *p*H the product gave an intense yellow color with *o*-aminobenzaldehyde. Since this color reaction is characteristic of Δ^1-pyrroline and related compounds,[18,19] the structure of the product was formulated as V; the yellow color is probably due to the formation of the dihydroquinazolinium compound (VI). V apparently was produced by spontaneous ring closure of II which, from the mode of synthesis, presumably was formed as an intermediate product. The carbon skeleton of V was confirmed by catalytic hydrogenation to DL-proline in 73% over-all yield. The reactive character of V is further illustrated by the fact that treatment with absolute ethanol at room temperature results in a biologically inactive product which may be the ethoxy compound (VII); the biological activity is largely recoverable on heating in water. V, like A, could not be isolated in a pure state, apparently due to the tendency of the pyrroline compound to polymerize. This reactivity and instability parallel the behavior of other aminoaldehyde derivatives.[18–20]

Evidence has been obtained that the excreted precursor A, presumably related to the L-series of amino-acids, is structurally identical with synthetic V. Culture filtrates from strain 55-1 containing A gave the characteristic yellow color with *o*-aminobenzaldehyde,[22] while control filtrates from 55-25 did not. Paper chromatography in two different solvents combined with the specific response of strain 55-25 yielded identical R_F values for A and V.

If the accumulation of precursor A by strain 55-1 is carried out in the presence of the relatively nontoxic *o*-aminobenzaldehyde, the yield by bio-assay of A as the dihydroquinazolinium compound (VI) is increased by a factor of five. The biological activity of VI is consistent with the observation that 1,2-dihydroquinazolinium compounds are somewhat dissociable into their parent compounds.[18,19] The increased output of A is probably due to the functioning of *o*-aminobenzaldehyde as an effective "trapping agent," causing the substantial removal from the system of A as such and a resulting shift in the equilibrium of the reaction sequence leading to A. The accumulation of A is also increased by addition of relatively large amounts of its precursor, glutamic acid.

The present results support the view that the

(16) A. M. Srb, J. R. S. Fincham and D. Bonner, *Am. J. Botany*, **37**, 533 (1950).

(17) M. Forbes and M. G. Sevag, *Arch. Biochem. Biophys.*, **31**, 406 (1951).

(18) C. Schöpf and F. Oechler, *Ann.*, **523**, 1 (1936).

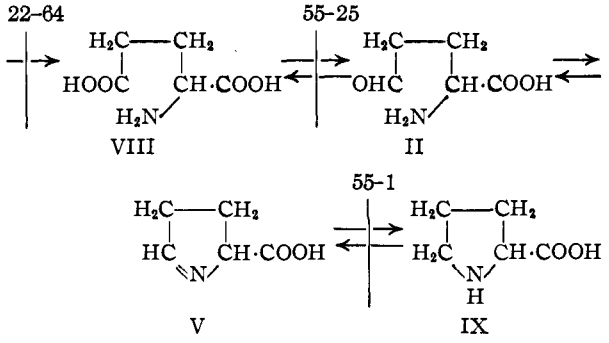
(19) C. Schöpf and H. Steuer, *ibid.*, **558**, 124 (1947).

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

(21) H. Tabor, *J. Biol. Chem.*, **188**, 125 (1951).

(22) In the visible region the absorption spectra of the reaction products of A and V with *o*-aminobenzaldehyde are approximately identical, but the comparison was complicated by the tendency of the color to increase in intensity on standing or on addition of a molar excess of *o*-aminobenzaldehyde. The latter compound apparently also gave rise to transformation products which made the results variable below 440 mμ.

path of synthesis of proline in *E. coli* includes reduction of glutamic acid (VIII) to glutamic γ-semialdehyde (II), cyclization to Δ^1-pyrroline-5-carboxylic acid V and further reduction to proline (IX), as shown in the diagram below. The reduction steps are presumably enzymatic, while the ring closure may be non-enzymatic since it is spontaneous *in vitro*.



V has also been found to be active without lag for certain proline auxotrophs of *Neurospora*.[23] The proposed path of proline synthesis is therefore not confined to *E. coli*, but may have rather general validity. It also seems possible that the biosynthesis of other heterocyclic compounds may involve similar reactions between amino and aldehyde groups.

Experimental

Organisms and Culture Media.—The organisms employed were mutants, obtained by the penicillin method,[24,25] of the W strain of *Escherichia coli* (ATCC 9637). For bacteriological tests and assays, the minimal medium described previously[26] (Medium A) was used. Solid media were prepared by the addition of 1.5% agar. For the accumulation of precursor A in liquid culture the following medium (Medium B) was used: KH_2PO_4, 0.3%; K_2HPO_4, 0.7%; $(NH_4)_2SO_4$, 0.1%; $MgSO_4 \cdot 7H_2O$, 0.0025%; glucose (autoclaved separately), 0.2%.

Stock cultures of the organisms, adequately stable for several months, were maintained in liquid Medium A supplemented with 0.2% casein digest (N-Z-Case, Sheffield) and stored at 3°.

Growth Response.—The mutants grew on solid or in liquid Medium A supplemented as follows: L-proline (strains 55-1, 55-25, 22-64); natural or synthetic Δ^1-pyrroline-5-carboxylic acid (strains 55-25, 22-64); L-glutamic acid (strain 22-64). Good growth was obtained with strains 55-1 and 55-25 on 10 to 20 γ/ml. of their respective supplements, while strain 22-64, which is blocked before glutamic acid, grew well on 100 to 200 γ/ml. of its supplements. No growth of any of the mutants occurred in Medium A without supplement or with 10 to 300 γ/ml. of racemic I[27] or III.

Accumulation of Precursor A.—Excretion of A was first indicated by the occurrence of syntrophism (cross-feeding) when strains 55-1 and 55-25 were streaked next to each other on Medium A containing limiting amounts of proline and incubated at 37°.[28] In a medium containing also 0.05% *o*-aminobenzaldehyde, a yellow zone was formed under and around the streak of strain 55-1.

In liquid culture, A was accumulated as follows: Medium B containing 10 γ/ml. of L-proline was inoculated with strain 55-1 and incubated with shaking at 37° for 24 to 48 hours. The resulting culture filtrate, when assayed as described below, contained an amount of A equivalent to 40 to 50 γ/ml. of L-proline. When 0.05% *o*-aminobenzaldehyde was incorporated into the medium, the deep yellow culture filtrate obtained assayed at an L-proline equivalent of 200 to 250 γ/ml. Addition of 1000 γ/ml. of L-glutamic acid increased the accumulation of A in the filtrate by about 50%.

Assay Methods.—Assays were carried out turbidimetrically using an Evelyn photoelectric colorimeter with a 620 mμ filter. One-ml. portions of 10 times strength Medium A (without glucose) were dispensed in calibrated Evelyn colorimeter tubes; the material to be assayed and water to make 10 ml. were added. Standard tubes containing 10 to 40 γ L-proline or 20 to 80 γ DL-proline per tube[29] and a control tube without added growth factor were similarly prepared. After sterilization by autoclaving at 120° for 15 minutes, the tubes were inoculated with 0.2 ml. of a bacterial suspension in 25% sterile aqueous glucose containing about 10^7 organisms per ml. and incubated at 37°. Strain 55-25 was used for assaying Δ^1-pyrroline-5-carboxylic acid or proline and 55-1 for proline. The optical densities were determined at 14 to 18 hours and again at 21 to 24 hours. Occasional phototrophic reversions causing misleading results were recognized by a sharp increase in density, and verified by subculture on solid minimal medium. The procedure is also applicable without modification to the assay of proline picrate and Compound VI.

Comparison of autoclaved and filter-sterilized samples showed not only that V withstood autoclaving, but that partly polymerized preparations had more activity after autoclaving.

The results of a typical assay are presented in Table I.

TABLE I

BIOASSAY WITH STRAIN 55-25

DL-Proline (γ/tube)	Unknown (ml./tube)[a]	Optical density[b]	Assay[c]
0	...	0.000	...
20	...	.056	...
40	...	.119	...
60	...	.177	...
80	...	.237	...
..	0.2	.063	111
..	.4	.137	115
..	.6	.194	110

[a] A neutral aqueous solution of synthetic V. [b] After incubation for 18 hours. [c] Expressed as γ DL-proline per ml. unknown solution; results obtained by interpolation; mean, 112 γ/ml.

Paper Chromatography Combined with Mutant Response.—Culture filtrates from strain 55-1 containing A were concentrated by distillation *in vacuo* and acidified with hydrochloric acid. Portions of the resulting solution and of hydrolysates of IV, prepared as described below, were chromatographed on Whatman #1 paper by the ascending method. The location of the active material on the chromatogram was determined by plating the dried paper on solid Medium A, seeded with about 10^8 organisms of strain 55-25 per ml., in flat glass trays. After incubation at 37° for 24 hours well-defined areas of growth revealed the position of active material.

The natural and synthetic products showed identical R_F values: 0.24 with a mixture of 77% *n*-butanol, 4.5% acetic acid and 18.5% water; 0.76 with 50% aqueous ethanol.

γ,γ-Dicarbethoxy-γ-acetamidobutyraldehyde (IV).—An ethanol solution of IV was prepared from acrolein and acetamidomalonic ester[30] as described by Moe and Warner.[31] The solvent was evaporated and the oily product was purified by adsorption on alumina (Merck, standardized according to Brockmann) from hexane–benzene (55:45) solution and elution with benzene–ether (50:50). After distillation of the solvents, IV was obtained as a colorless, viscous oil. Alternatively IV was purified *via* its phenylhydrazone. One gram of recrystallized phenylhydrazone,[31] 0.2 ml. of glacial acetic acid, 3 ml. of freshly distilled benzaldehyde, and 20

(23) These tests were kindly performed by Dr. A. M. Srb.

(24) B. D. Davis, THIS JOURNAL, **70**, 4267 (1948); *Proc. Natl. Acad. Sci., U. S.*, **35**, 1 (1949).

(25) J. Lederberg and N. Zinder, THIS JOURNAL, **70**, 4267 (1948).

(26) B. D. Davis and E. S. Mingioli, *J. Bact.*, **60**, 17 (1950).

(27) Generously furnished by Dr. N. H. Horowitz.

(28) For illustration of syntrophism, *cf.* ref. 15.

(29) L-proline shows twice the activity by weight of DL-proline.

(30) We are indebted to Dr. O. A. Moe for a gift of ethyl acetamidomalonate.

(31) O. A. Moe and D. T. Warner, THIS JOURNAL, **70**, 2763 (1948).

ml. of 95% ethanol were refluxed for 15 minutes and distilled to dryness *in vacuo*. The residue was dissolved as far as possible in 15 ml. of water, and the resulting aqueous solution was filtered from the benzaldehyde phenylhydrazone formed, treated with activated charcoal, and evaporated to dryness *in vacuo*, yielding IV as a colorless oil.

Δ^1-Pyrroline-5-carboxylic Acid (V).—A solution of 273 mg. (1.0 millimole) IV in 5 ml. of 6 *N* hydrochloric acid was refluxed for 15 minutes. A portion of the resulting hydrolysate was diluted, neutralized with potassium carbonate, and immediately assayed with strain 55-25. The total activity found was very nearly equivalent to that of 1 millimole DL-proline. The yield of V was thus almost quantitative, if it is assumed that V is not more active than DL-proline. The hydrolysate was completely inactive for strain 55-1, showing absence of proline. The volatile hydrolysis products could be removed by distilling to dryness *in vacuo*.[32] Hydrolysates of IV purified *via* its phenylhydrazone were clear and colorless, while unpurified IV gave dark brown solutions; however, even such crude hydrolysates were adequate to demonstrate the growth response of the mutants. Crude IV gave 75 to 90% yields of V. In one experiment using crude IV the yield of V was studied as function of refluxing time; after 15 and 30 minutes, the yield was 90%, after 2 hours, 40%, and after 8 hours, 20%. Some proline was formed on prolonged heating, as shown by the response of strain 55-1. Hydrolysates of IV retained most of their biological activity for several weeks when stored at 3°; biological activity lost was partly recovered when the product was autoclaved at *p*H 7 in the minimal medium described.

Hydrogenation of Δ^1-Pyrroline-5-carboxylic Acid (V).—A hydrolysate, prepared by the method described from 300 mg. IV was evaporated to dryness *in vacuo*. The residue was immediately dissolved in 5 ml. of 80% aqueous acetic acid and shaken in hydrogen with 30 mg. Adams catalyst at 25° and atmospheric pressure until no more hydrogen was taken up. The reaction mixture was filtered from the catalyst and distilled to dryness. Assay of the resulting residue with strain 55-1 showed that a yield of 92 mg. of DL-proline (73% on the basis of IV) had been obtained. Assay with strain 55-25 gave the same yield, showing absence of V in the hydrogenated product. The formation of proline was confirmed by isolation as picrate.

DL-Proline Picrate.—The hydrogenated product described above, dissolved in water, was freed of chloride by successive treatment with silver carbonate and hydrogen sulfide. The resulting solution was treated with charcoal (Darco G-60) and evaporated to dryness. The residue was dissolved as far as possible in 10 ml. of 95% ethanol. The resulting solution was filtered from some undissolved material and distilled to dryness. The crystalline residue and 180 mg. of picric acid were dissolved in hot glacial acetic acid. The resulting product, after recrystallization from glacial acetic acid, weighed 140 mg. and melted at 136°.

Anal. Calcd. for $C_{11}H_{12}O_9N_4$: C, 38.38; H, 3.51; N, 16.28. Found: C, 38.35; H, 3.42; N, 16.01.

A mixed melting point with authentic DL-proline picrate[33] was undepressed and bioassay of the product and authentic picrate with strain 55-1 showed identical proline contents equal to the expected amount of about 33%.

Acknowledgments.—We are indebted to Roscoe C. Funk, Jr., Sloan-Kettering Institute for Cancer Research, for the microanalyses reported. The excellent technical assistance of Elizabeth S. Mingioli is gratefully acknowledged.

(32) The resulting residue or its aqueous solution were less stable than the original hydrolysate.

(33) D. Alexandroff, *Z. physiol. Chem.*, **46**, 17 (1905).

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[CONTRIBUTION FROM THE EDWARD MALLINCKRODT DEPARTMENT OF PHARMACOLOGY OF WASHINGTON UNIVERSITY SCHOOL OF MEDICINE]

Enzymatic Synthesis of Desoxyxanthosine by the Action of Xanthosine Phosphorylase in Mammalian Tissue[1,2]

BY MORRIS FRIEDKIN

Desoxyxanthosine, the desoxyribosidic analog of xanthosine, has been isolated and characterized as the cyclohexylamine salt. The new purine desoxyriboside was formed enzymatically from xanthine and desoxyribose-1-phosphate by the action of xanthosine phosphorylase present in rat liver preparations. An analogous synthesis of xanthosine was carried out with xanthine and ribose-1-phosphate. The phosphorolysis of xanthosine and desoxyxanthosine occurs at a much slower rate than the phosphorolysis of desoxyguanosine.

Although xanthosine (xanthine riboside) has been known for some time,[3] the desoxyribosidic analog has not been described. During the course of experiments designed to increase the yield of desoxyribose-1-phosphate formed by the enzymatic phosphorolysis of desoxyguanosine[4] it was noticed that xanthine, one of the reaction products, unexpectedly disappeared with a concomitant loss of desoxyribose-1-phosphate. These observations lead to the isolation of the hitherto undescribed nucleoside: desoxyxanthosine (xanthine desoxyriboside) which was formed by an enzymatic reaction between xanthine and desoxyribose-1-phosphate as shown in reaction (1).

A completely analogous reaction between xanthine and ribose-1-phosphate with the formation of xanthosine was also shown to occur.

Indirect Enzymatic Formation of Desoxyxanthosine from Desoxyguanosine.—The enzyme solutions used in the present study were known from previous studies to catalyze reactions (2) and (3).[4] Desoxyguanosine + inorganic P $\rightleftarrows$ guanine + desoxyribose-1-phosphate (2). Guanine $\rightarrow$ xanthine (3)

Despite the reversibility of reaction (2) a good yield of desoxyribose-1-phosphate was predicted because of the probable irreversibility of reaction (3).[5] Thus for each mole of desoxyguanosine which undergoes enzymatic phosphorolysis, one mole of xanthine should also be formed owing to

(1) Presented before the Division of Biological Chemistry at the 118th Meeting of the American Chemical Society, Chicago, Ill., September, 1950.

(2) This investigation was supported (in part) by a research grant from the National Institutes of Health, Public Health Service.

(3) P. A. Levene and W. A. Jacobs, *Ber.*, **43**, 3150 (1910); W. Jones, *J. Biol. Chem.*, **9**, 169 (1911); S. J. Thannhauser and B. Ottenstein, *Z. physiol. Chem.*, **114**, 2 (1921).

(4) M. Friedkin and H. M. Kalckar, *J. Biol. Chem.*, **184**, 437 (1950).

(5) This is an assumption which may not be true. The reversibility of guanine deaminase activity appears never to have been fully studied.