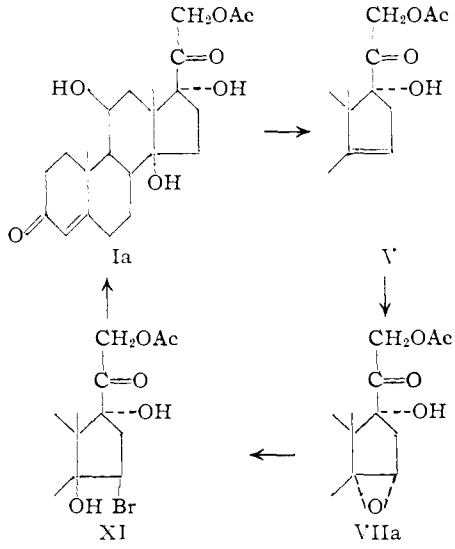

infrared region. These reactions unequivocally demonstrated the presence in (I) of a non-acetylatable secondary alcohol grouping (11β-hydroxyl) as well as a tertiary hydroxyl function.

With the elucidation of the gross structural features of (I), allocation of the tertiary hydroxyl function to the 14α-position followed from the exact parallelism observed between the chemical reactions of (I) and those of authentic Δ^4-pregnene-14α,17α,21-triol-3,20-dione (14α-hydroxy-Compound S).[4] Demonstration of the presence of the 14α-hydroxyl substituent allowed complete formulation of (I) as Δ^4-pregnene-11β,14α,17α,21-tetrol-3,20-dione and of (II) as Δ^4-pregnene-14α,17α,21-triol-3,11,20-trione.[5]

Treatment of (Ia) with *p*-toluenesulfonic acid in refluxing benzene resulted in the selective removal of the tertiary hydroxyl group to give $\Delta^{4,14}$-pregnadiene-11β,17α,21-triol-3,20-dione acetate (V), m.p. 253–255° dec., $[\alpha]_D$ +116° (dioxane), λ_{max}^{alc} 242 mμ (log ϵ 4.21); Found: C, 68.5; H, 7.56; which was oxidized with chromic acid to $\Delta^{4,14}$-pregnadiene-17α,21-diol-3,11,20-trione acetate (VI), m.p. 200–201°, $[\alpha]_D$ +121° (dioxane), Found: C, 68.9; H, 6.86. Oxidation of (V) with excess monoperphthalic acid gave Δ^4-14α,15α-oxidopregnene-11β,17α,21-triol-3,20-dione acetate (VIIa), m.p. 229–230°, $[\alpha]_D$ +144° (dioxane), λ_{max}^{alc} 240 mμ (log ϵ 4.21); Found: C, 65.7; H, 7.14; the 21-alcohol (VII) of which had m.p. 225–226°, $[\alpha]_D$ +159° (dioxane), λ_{max}^{alc} 239 mμ (log ϵ 4.23); Found: C, 66.6; H, 7.45. As expected, (VIIa) was oxidized with chromic acid to the corresponding Δ^4-14α,15α-oxidopregnene-17α,21-diol-3,11,20-trione acetate (VIII), m.p. 184–186°, $[\alpha]_D$ +186° (dioxane), λ_{max}^{alc} 237 mμ (log ϵ 4.22); Found: C, 66.3; H, 6.80.

D-Ring halogen-substituted derivatives of (I) were prepared by the same method utilized in the 11-desoxy series.[4] When the oxido derivatives (VIIa) and (VIII) were treated with hydrogen chloride in chloroform at 0°, the products were Δ^4-15β-chloropregnene-11β,14α,17α,21-tetrol-3,20-dione acetate (IX), m.p. 174–175°, $[\alpha]_D$ +110° (dioxane), λ_{max}^{alc} 240 mμ (log ϵ 4.22), Found: C, 60.0; H, 6.93; Cl, 7.82; and Δ^4-15β-chloropregnene-14α,17α,21-triol-3,11,20-trione acetate (X), m.p. 233–234° dec., $[\alpha]_D$ +106° (dioxane), λ_{max}^{alc} 237 mμ (log ϵ 4.21), Found C, 60.9; H, 6.42; Cl, 7.91; respectively.

The reaction of (VIII) with hydrogen bromide in chloroform at −15° afforded Δ^4-15β-bromopregnene-14α,17α,21-triol-3,11,20-trione acetate (XI), m.p. 207–209° dec., $[\alpha]_D$ +118° (dioxane), λ_{max}^{alc} 237 mμ (log ϵ 4.21), Found: C, 55.4; H, 5.87. On similar treatment the 11β-hydroxylated epoxide (VIIa) resulted in a markedly unstable bromohydrin (Δ^4-15β-bromopregnene-11β,14α,17α,21-tetrol-3,20-dione acetate) (XII), m.p. 129–131° dec., which could be converted back to (Ia) by dehalogenation with Raney nickel,[6] thereby establishing the stereochemistry of the above-mentioned epoxides and halohydrins.

(4) B. M. Bloom, E. J. Agnello and G. D. Laubach, in preparation.

(5) The structures assigned to (I) and (II) were further substantiated by oxidative side-chain cleavage experiments of (I) with chromic acid and sodium bismuthate. The chromic acid product (III) had the molecular formula $C_{19}H_{24}O_4$, m.p. 283–285° dec., $[\alpha]_D$ +208° ($CHCl_3$), λ_{max}^{alc} 236.5 mμ (log ϵ 4.20), Found: C, 72.3; H, 7.88; and infrared spectrum consistent with its formulation as Δ^4-androsten-14α-ol-3,11,17-trione. Sodium bismuthate cleavage of (I) afforded a product (IV), $C_{19}H_{26}O_4$, which exhibited m.p. 224–226°, $[\alpha]_D$ +169° (dioxane), +186° ($CHCl_3$), λ_{max}^{alc} 241 mμ (log ϵ 4.20), Found: C, 72.0; H, 8.28; and infrared spectrum consistent with its structural assignment as Δ^4-androstene-11β,14α-diol-3,17-dione.

(6) P. L. Julian, *et al.*, THIS JOURNAL, **72**, 5145 (1950).

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BIOSYNTHESIS OF NUCLEIC ACID GUANINE: THE ENZYMIC CONVERSION OF INOSINE-5′-PHOSPHATE TO XANTHOSINE-5′-PHOSPHATE[1]

Sir:

The work of several investigators implicates inosine-5′-phosphate as a precursor of nucleic acid purines.[2,3] We wish to report its conversion to xanthosine-5′-phosphate.

Sonic extracts of *Aerobacter aerogenes*, strain 1033, were precipitated with half-saturated acidic ammonium sulfate, dialyzed, and treated with protamine sulfate. The protamine supernate reduced DPN but not TPN in the presence of inosine-5′-phosphate, but not in the presence of inosine or hypoxanthine. The reaction, followed spectrophotometrically at 340 mμ, proceeded without lag under anaerobic conditions at *p*H 8.3 and required NH_4^+ and cysteine for maximum activity. A fourfold purification was achieved by collecting the protein precipitated between 20–30% ammonium sulfate saturation.

To obtain sufficient product for characterization, an extract of glycerol-grown cells was used and the DPNH reoxidized by dihydroxyacetone through the glycerol dehydrogenase present. The reaction

(1) This work was supported in part by an institutional grant to Harvard University from the American Cancer Society, by a research grant (G-3554) from the U. S. Public Health Service, and by funds received from the Eugene Higgins Trust.

(2) R. G. Greenberg, THIS JOURNAL, **74**, 6307 (1952).

(3) J. M. Buchanan and M. P. Schulman, *J. Biol. Chem.*, **202**, 241 (1953).

mixture contained 0.6 ml. of the 20–30% $(NH_4)_2SO_4$ fraction (5.6 mg. of protein), 17.6 micromoles of inosine-5′-phosphate, 5.4 micromoles of DPN, 30 micromoles of dihydroxyacetone, 10.8 micromoles of cysteine and 90 micromoles of $(NH_4)_2SO_4$ in a total volume of 9 ml. of 0.01 *M* glycylglycine buffer, *p*H 8.3. After incubation at 37° for one hour, the mixture was deproteinized with chloroform–octanol and separated on a Dowex-2 (200–400 mesh)-acetate column[4] (11.5 cm. × 2.5 sq. cm.) using gradient elution with ammonium acetate solution (initial concentration in reservoir 0.6 *M*, *p*H 6.0, with a solution 1 *M*, *p*H 4.5 added dropwise during elution). Three separate bands of ultraviolet-absorbing material were eluted, having 250/275 mμ ratios of 1.9, 3.5, and 1.0, respectively. The first band was shown by paper chromatography to contain DPN and some inosine and hypoxanthine. The second band consisted of 9.8 micromole of inosine-5′-phosphate, identified by its position and spectrum. The compound present in the third band was identified as xanthosine-5′-phosphate (Table I); 0.82 mole per mole of inosine-5′-phosphate used was formed.

TABLE I

CHARACTERIZATION OF PRODUCT AS XANTHOSINE-5′-PHOSPHATE

Ultraviolet extinction, mμ →	Maximum	Minimum
Product, *p*H 7	248, 277	265
Xanthosine, *p*H 7	248, 278	265
Hydrol. product, *p*H < 1	261	243
Xanthine, *p*H < 1	260	242

Electrophoretic mobility on paper at *p*H 7 relative to inosine-5′-phosphate

	Found	Calculated
Product	1.55	1.50

Rf in	KH_2PO_4: isoamyl alcohol	butanol: NH_3: diethylene-glycol
Hydrolyzed product	0.48	0.12
Xanthine	0.49	0.12

Composition	mole per mole[a] Found	Theoretical
Xanthine	0.96	1
Pentose	1.0	1
Phosphate	1.04	1
Periodate reactive	1.06	1

[a] Calculated from ultraviolet at 275 mμ as xanthosine.

The accumulation of xanthosine by a guanine-less auxotroph of this organism had suggested the mutant to be locked in the conversion of a derivative of xanthine to one of guanine.[5] The present results appear to identify this xanthine derivative as xanthosine-5′-phosphate and indicate the DPN-linked conversion of inosine-5′-phosphate to xanthosine-5′-phosphate to be a step in the biosynthesis of nucleic acid guanine.

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RECEIVED JULY 18, 1955

(4) The use of Dowex-acetate was suggested by Dr. Marian Bentley of Montefiore Hospital, Pittsburgh, Pennsylvania.

(5) B. Magasanik and M. S. Brooke, *J. Biol. Chem.*, **206**, 83 (1954).

ACCUMULATION OF DIMETHYLPYRUVIC ACID IN BIOTIN-DEFICIENT CULTURE OF *PIRICULARIA ORYZAE*

Sir:

During the course of studies[1,2] on the metabolic function of biotin using the rice-blast fungus, *Piricularia oryzae* Cavara strain No. 1, which requires biotin and thiamine as growth factors, it has been found that dimethylpyruvic acid (DMPA) was accumulated in biotin-deficient culture.

In biotin-deficient media[1] (5 mγ/20 ml.), this fungus grew so slowly that it required 30 days to reach its maximum growth, and the *p*H of media decreased from 6.6 to 4.6 during the first 13 days of cultivation due to the accumulation of a large concentration of organic acids. On the contrary, in biotin-rich media (100 mγ/20 ml.), the growth of the fungus was rapid and the *p*H decreased little.

The biotin-deficient culture fluid was separated from mycelia after 13 days and treated with 2,4-dinitrophenylhydrazine in 4*N* HCl. After standing overnight in an ice-box the precipitated 2,4-dinitrophenylhydrazones were filtered, converted to ammonium salts and separated by chromatography on alumina. After the neutral hydrazones had been passed through an alumina column, acidic hydrazones were found to give 4 bands (A_1, A_2, A_3, and A_4). Each band was eluted with ammoniacal methanol and the free hydrazone, obtained by decomposing the ammonium salt with HCl, was recrystallized twice from 50% ethanol. The yield and the melting point of each hydrazone is shown in Table I.

TABLE I

YIELDS AND MELTING POINTS OF THE HYDRAZONES

	A_1	A_2	A_3	A_4	Neutral hydrazones
Yield per 1 l. of culture media, mg.	82	232	93	830	175
M.p., °C.	219°	217°	211°	184°	

A_1 and A_2 were identified as the hydrazone of α-ketoglutaric acid (KGA) and the *anti*-isomer[3] of the hydrazone of pyruvic acid (PA), respectively, by their melting points. A_3 was shown to be the *syn*-isomer[3] of the hydrazone of PA, and A_4 the hydrazone of DMPA by means of analyses and by the failure of A_4 to depress the melting point of an authentic sample.[4] A_3: *Anal.* Calcd. for $C_9H_8O_6N_4$: C, 40.31; H, 3.01; N, 20.89. Found: C, 40.53; H, 2.97; N, 21.06; A_4: *Anal.* Calcd. for $C_{11}H_{12}O_6N_4$: C, 44.59; H, 4.08; N, 18.92; Found: C, 44.90; H, 4.03; N, 18.95.

Paper chromatographic comparison[5] of the natural DMPA (A_4) with an authentic sample in an *n*-butanol–petroleum ether (4:1) system showed that they were identical (R_f 0.68 and 0.73, respectively). R_f values of the other acids also agreed with known[5] values (A_1, 0.02; A_2, 0.13; A_3, 0.25).

(1) H. Katsuki, *J. Chem. Soc. Japan*, **76**, in press (1955).

(2) H. Katsuki, *ibid.*, in press.

(3) T. Moriwaki, H. Katsuki and S. Tanaka, *J. Chem. Soc. Japan*, in press; F. A. Isherwood and R. L. Jones, *Nature*, **175**, 419 (1955).

(4) The author is grateful to Mr. K. Imai for supplying the authentic sample of dimethylpyruvic acid.

(5) D. Cavallini, N. Frontali and G. Toschi, *Nature*, **163**, 568 (1949).