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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(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).

The calculated molar ratios of these keto-acids are: KGA:PA:DMPA = 0.25:1.2:2.8.

The accumulation of these keto-acids was not due to acidification of the media, because they also occurred in biotin-deficient culture with calcium carbonate added. On the contrary, no accumulation of keto-acids occurred in biotin-rich media, or when preformed biotin-rich mycelial felts were floated on media adjusted to *p*H 4.6 with phosphoric acid. In thiamine-deficient culture, the accumulation of PA and KGA was observed but no DMPA could be found. The accumulation of DMPA is, therefore, to be attributed to biotin-deficiency.

Grateful acknowledgment is made to Prof. S. Tanaka for his interest and encouragement.

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REVERSIBLE ISOMERIZATIONS IN THE TETRACYCLINE FAMILY

Sir:

We have recently observed a reversible isomerization reaction creating for each of four members of the tetracycline family—chlorotetracycline,[1] bromotetracycline, oxytetracycline,[2] and tetracycline[3]—a new, isomeric substance. For each of the four tetracycline family members above, sets of conditions have been found catalyzing the formation of an equilibrium mixture of two components. For example, in the case of tetracycline itself, a twenty-four hour aging at 25° of a 15% tetracycline solution in 1 molar NaH_2PO_4 in 2:1 methanol–water (*p*H about 4.6) produced an equilibrium mixture judged spectrophotometrically and microbiologically to be about a 1.5:1 mixture of tetracycline and its new isomer, designated quatrimycin. Quatrimycin was isolated from the equilibrium mixture as the crystalline, homogeneous ammonium salt. Quatrimycin differs greatly in some of its properties from the starting tetracycline. For example, its isoelectric form is more water soluble. Its *in vitro* antibiotic activity against a variety of tetracycline-susceptible microörganisms is substantially less than that of tetracycline; for example, toward the turbidimetric assay using *E. coli*, quatrimycin shows 2–5% the activity of tetracycline. The possibility exists that the *in vitro* activity is actually zero, with partial equilibration under the test conditions accounting for the observed bioactivity. Re-equilibration of the isolated quatrimycin under the conditions used for tetracycline resulted in a reappearance of *in vitro* antibiotic activity and alteration in the ultraviolet absorption spectrum until the approximately 1.5:1 equilibrium mixture was again attained, from which both tetracycline and quatrimycin were re-isolated. The ultra violet absorption spectra, in 0.1 *N* HCl, for tetracycline and quatrimycin are presented in the graph.

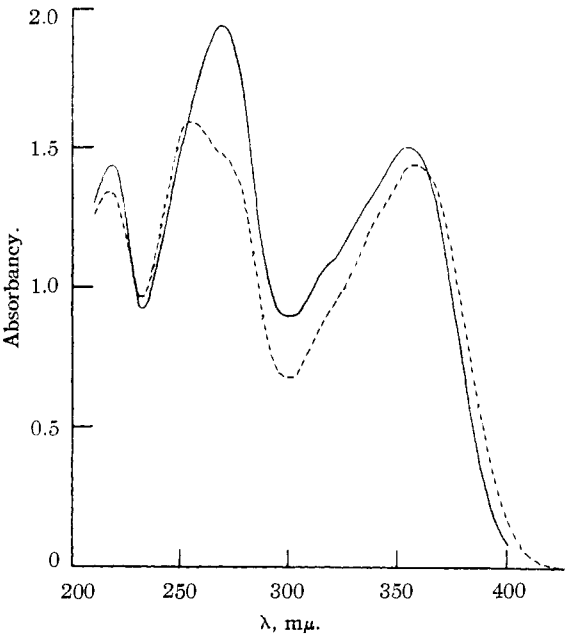

Fig. 1.—Tetracycline hydrochloride, 50.0 mg./l. in 0.1 *N* H_2SO_4——; quatrimycin, ammonium salt, 50.6 mg./l. in 0.1 N. H_2SO_4- - -.

Similarly, the equilibration of either tetracycline or quatrimycin can be accomplished in various buffers, such as formate, acetate or citrate, or in distilled water if sufficient time is allowed. The equilibrium can also be attained in various organic solvent systems, such as methanol. The solid crystalline materials are stable.

A similar series of observations holds for chlorotetracycline, bromotetracycline, and oxytetracycline. Their new isomers, chloroquatrimycin, bromoquatrimycin, and oxyquatrimycin, are all of lowered *in vitro* antibiotic activities and are changed in their ultraviolet spectra in the manner described for tetracycline. Preliminary animal work[4] shows each of the four new isomers to possess broad *in vivo* antibiotic activity.

(1) The trademark of the American Cyanamid Company for chlorotetracycline is Aureomycin.

(2) The trademark of Charles Pfizer and Company for oxytetracycline is Terramycin.

(3) The trademark of the American Cyanamid Company for tetracycline is Achromycin. The trademark of Charles Pfizer and Company for tetracycline is Tetracyn.

(4) This work was done under the direction of Dr. J. S. Kiser, Research Division, American Cyanamid Company.

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ON THE STEREOCHEMISTRY OF RESERPINE

Sir:

In a recent communication[1] we presented evidence for the relative and absolute configuration of four asymmetric centers (15, 16, 18 and 20) in reserpine (I).

(1) P. A. Diassi, F. L. Weisenborn, C. M. Dylion and O. Wintersteiner, THIS JOURNAL, **77**, 2028 (1955).