At 275° and 5 mm. pressure Φ C_4H_8, was 0.32 compared to 0.22, 0.13, 0.010 and 1.15 for the quantum yields of methane, propylene, ethane and carbon monoxide, respectively. Apparently, no significant amounts of butene were formed by a direct primary process, or by a combination reaction between methyl and propenyl radicals. The chain sequence, (1)–(2), initiated by methyl radicals formed in one or more primary photochemical processes can explain the relatively large yields of butene and the fact that Φ CO exceeds unity.

B. Photolysis of a *trans*-Methyl Propenyl Ketone–Acetone Mixture at 2654 Å. and 275°.—In the photolysis of this mixture at 2654 Å. the acetone acted as an excellent source of methyl radicals and it was found that at 275° over seven times as much 2-butene was formed as in the photolysis of the pure unsaturated ketone. Further evidence for the chain process (1)–(2) is the fact that Φ CO was 1.43 for the mixture, *vs.* 1.00 for acetone[3] and 0.50 for *trans*-methyl propenyl ketone.

C. Pyrolyses of Mixtures of *trans*-Methyl Propenyl Ketone and Di-*t*-butyl Peroxide.—Mixtures of di-*t*-butyl peroxide and *trans*-methyl propenyl ketone were heated in the dark at 170 and 150°, respectively, for about one half-life of the peroxide. Under these conditions the primary process in the pyrolysis of di-*t*-butyl peroxide is known to give methyl radicals and acetone.[4] The results are summarized in Table I.

TABLE I

VOLUMES OF NON-CONDENSABLE PRODUCTS[a] FROM PYROLYSES OF DI-*t*-BUTYL PEROXIDE–*trans*-METHYL PROPENYL KETONE MIXTURES

	CO	C_4H_8	CH_4	C_2H_6
[Peroxide (17 mm.) + ketone (79 mm.)] at 170° for 22 min.	0.26	0.25	0.29	0.41
[Peroxide (18 mm.) + ketone (86 mm.)] at 150° for 183 min.	.26	.26	.42	.11

[a] Values given are volumes of products (cc. at 25° and 750 mm.) per half-life (approximate) of peroxide decomposed.

From these data it is clear that reaction (1) does not require "hot" methyls generated by photolytic methods but instead this process is also efficient with "thermal" methyl radicals at 150°. Furthermore, the equivalence of carbon monoxide and 2-butene is excellent evidence that they are generated in the same reaction sequence, namely (1) and (2). No propylene was formed in the pyrolyses of these mixtures.

It can be shown from material balances that a large percentage of methyl radicals must add to the carbon–carbon double bond in such a way that stable condensable products are ultimately formed. In accord with this idea qualitative mass spectrometric evidence was obtained for methyl isobutyl ketone as a photolysis product in A. Such a product can be rationalized on the basis that β-addition of methyl to the carbon–carbon double bond gives a relatively stable allylic type radical which abstracts a hydrogen atom from the substrate to give the saturated ketone, whereas α-addition gives a less stable radical that dissociates into 2-butene and acetyl.

Further work is now in progress to investigate the generality[5] and possible stereospecificity of this type of reaction.

(3) W. A. Noyes, Jr., and L. M. Dorfman, *J. Chem. Phys.*, **16**, 788 (1948).

(4) J. H. Raley, F. F. Rust and W. E. Vaughan, THIS JOURNAL, **70**, 88, 1336, 2767 (1948).

(5) F. E. Blacet and W. E. Bell, *Discs. Faraday Soc.*, **70** (1953), present evidence for a process somewhat analogous to (1), that is, methyl radical attack on biacetyl to give acetone and acetyl radical.

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ENZYMATIC SYNTHESES OF PYRIMIDINE AND PURINE NUCLEOTIDES. II.[1] OROTIDINE-5'-PHOSPHATE PYROPHOSPHORYLASE AND DECARBOXYLASE

Sir:

Orotic acid is known to be a precursor of nucleic acid and nucleotide pyrimidines in certain bacteria[2] and animal tissues[3] but the pathway of the conversion requires clarification. We have studied a pathway of orotic acid utilization by pigeon liver enzymes in which ribose-5-phosphate (R5P) and adenosine triphosphate are required.[4] These substances, in the presence of a partially purified enzyme, produce 5'-phosphoribosylpyrophosphate (PRPP). We proposed a condensation of this new ester with orotic acid (or adenine) to produce orotidine-5'-phosphate (O5P) (or adenosine-5'-phosphate (A5P)) and pyrophosphate (PP).[5] With enzymes purified from yeast, we have observed the catalysis of O5P formation by a reversible mechanism (equation 1) and the decarboxylation of O5P to uridine-5'-phosphate (U5P) (equation 2). The enzymes, respectively, are O5P pyrophosphorylase and O5P decarboxylase.

$$\text{Orotic acid} + \text{PRPP} \rightleftharpoons \text{O5P} + \text{PP} \qquad (1)$$

$$\text{O5P} \longrightarrow \text{U5P} + CO_2 \qquad (2)$$

The stoichiometry of the over-all reaction starting with orotic acid and PRPP (equations 1 and 2) was demonstrated with a relatively crude enzyme fraction (I) which contained both O5P pyrophosphorylase and O5P decarboxylase (Table I). Fluoride was added to inhibit a very active PPase also present. In a separate experiment, carried out under similar conditions, 3.43 μmoles of 4,7-C^{14}-orotic acid (51,500 c.p.m./μmole) were utilized and 3.04 μmoles of U5P was isolated. The U5P was characterized on the basis of its absorption spectrum ($\lambda_{230}/\lambda_{260}$ = 0.36 at *p*H 3.2), its properties on ion-exchange chromatography (eluted from Dowex 1 formate, 10% cross-linked, between 8 and 18 resin bed volumes of 0.1 *M* formate, *p*H 3.2, peak at 12 volumes), and by the release of 2.86 μmoles of phos-

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

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(4) I. Lieberman, A. Kornberg, E. S. Simms and S. R. Kornberg, *Federation Proc.*, **13**, 252 (1954).

(5) A. Kornberg, I. Lieberman and E. S. Simms, THIS JOURNAL, **76**, 2027 (1954).

TABLE I

STOICHIOMETRY OF THE CONDENSATION OF OROTIC ACID AND PRPP

The reaction mixture (24 ml.) contained 0.48 ml. of tris-(hydroxymethyl)-aminomethane (TRIS) buffer (1 *M*, *p*H 8.0), 0.48 ml. of $MgCl_2$ (0.1 *M*), 1.20 ml. of PP (0.01 *M*, *p*H 8.5), 1.44 ml. of NaF (0.5 *M*) 0.48 ml. of 4,7-C^{14}-sodium orotate (0.01 *M*, 186,000 c.p.m./μmole), 0.40 ml. of PRPP (2.85×10^{-3} *M*, 112,000 c.p.m./μmole) and 4.8 ml. of enzyme fraction I (containing 20.6 mg. of protein). One-half of the reaction mixture was immediately placed in a boiling water-bath for 0.5 min.; the remainder was incubated at 30° for 40 minutes and then heated in a boiling water-bath for 0.5 min.

	μmoles		
	0 min.	40 min.	Δ
Orotic acid[a]	2.40 (2.38)[e]	1.84 (1.87)	−0.56 (−0.51)
PRPP[b]	0.57 (0.52)	0.03 (0.00)	−0.54 (−0.52)
U5P[c]	.00 (0.00)	.55 (0.55)	+ .55 (+0.55)
CO_2[d]		.60	+ .60
PP	(0.09)	(0.48)	(+0.39)[f]

[a] Determined spectrophotometrically at 295 mμ. [b] Determined spectrophotometrically by the removal of orotic acid (see equation 1). [c] Determined spectrophotometrically at 260 mμ. [d] Estimated by trapping in NaOH the $C^{14}O_2$ released enzymatically from orotic acid and measuring the radioactivity. [e] Values in parentheses were determined after chromatography on Dowex 1 anion-exchange resin; orotic acid and U5P were estimated by optical density measurements at 280 and 262 mμ, respectively; PRPP and PP were estimated by radioactivity measurements. [f] This value is low because of inorganic PPase activity; 0.22 μmole was recovered as 0.44 μmole of inorganic orthophosphate.

phate by 5'-nucleotidase.[6] The specific activity of the U5P (24,700 c.p.m./μmole) was one-half that of the orotic acid.

The accumulation of O5P was demonstrated with a more purified enzyme preparation (II) which was free of decarboxylase and relatively poor in PPase. In an experiment in which 0.27 μmole of 4,7-C^{14}-orotic acid was consumed, 0.26 μmole of O5P was isolated by ion-exchange chromatography. The O5P was identified by its absorption spectrum (identical with that of orotidine[7]: peak at 266 mμ, $\lambda_{280}/\lambda_{260} = 0.66$ at *p*H 7), and its enzymatic decarboxylation (0.26 μmole $C^{14}O_2$ liberated; equation 2). The synthesis of larger amounts of O5P is made difficult by the rapid decline in reaction rate due to the accumulation of O5P and PP.

The stoichiometry of the O5P pyrophosphorylase reaction (equation 1) with O5P (prepared by phosphatase transfer[8]) and PP as substrates was observed with the results shown in Table II. In the presence of larger amounts of PP the reaction proceeds rapidly to completion. Thus, in a 1-ml. incubation mixture containing partially purified O5P pyrophosphorylase (0.02 mg. of protein), 0.036 μmole of O5P and 0.30 μmole of PP, 0.037 μmole of orotic acid was formed in 17 minutes. No reaction occurred in the absence of added PP.

(6) L. A. Heppel and R. J. Hilmoe, *J. Biol. Chem.*, **188**, 665 (1951).

(7) A. M. Michelson, W. Drell and H. K. Mitchell, *Proc. Natl. Acad. Sci.*, **37**, 396 (1951).

(8) A compound having an absorption spectrum identical to O5P synthesized by the enzymatic condensation of orotic acid and PRPP has been prepared from orotidine by phosphate transfer from phenylphosphate with a malt phosphatase (G. Brawerman and E. Chargaff, THIS JOURNAL, **75**, 2020 (1953)). It was further identified as O5P by its properties on ion-exchange chromatography, by its enzymatic decarboxylation to U5P (0.061 μmole yielded 0.061 μmole of U5P estimated spectrophotometrically), and by the molar ratios of orotic acid:pentose:phosphate of 1.08:1.04:1.06.

TABLE II

STOICHIOMETRY OF THE PYROPHOSPHORLYSIS OF O5P

The reaction mixture (21.6 ml.) contained 0.36 ml. of TRIS buffer (1 *M*, *p*H 8.0), 0.36 ml. of $MgCl_2$ (0.1 *M*), 1.8 ml. of NaF (0.05 *M*), 1.8 ml. of PP (1.2×10^{-3} *M*, 750,000 c.p.m./μmole), 13.2 ml. of O5P (9.8×10^{-5} *M*) and 3.6 ml. of the enzyme fraction II (0.42 mg. of protein). One half of the reaction mixture was immediately placed in a boiling water-bath for 0.5 min.; the other half was incubated at 30° for 22 min. and then placed in a boiling water-bath for 0.5 min.

	μmoles		
	0 min.	22 min.	Δ
O5P[a]	0.65 (0.58)[d]	0.39 (0.29)	−0.26 (−0.29)
PP	(1.04)	(0.84)	(−0.20)
Orotic acid[b]	.00 (0.00)	.32 (0.30)	+ .32 (+0.30)
PRPP[c]	.00 (0.00)	.22 (0.16)	+ .22 (+0.16)

[a] Estimated spectrophotometrically at 266 mμ. [b] Determined by the decrease in optical density at 280 mμ in the presence of dihydroörotic dehydrogenase and reduced diphosphopyridine nucleotide (I. Lieberman and A. Kornberg, *Biochim. Biophys. Acta*, **12**, 223 (1953)). [c] See footnote *b*, Table I. [d] See footnote *e*, Table I. The relatively low value at 22 min. for PRPP and the high value for PP at 22 min. suggest the destruction of PRPP to yield PP.[5]

O5P pyrophosphorylase did not convert adenine or uracil to their respective nucleotides in detectable amounts (8-C^{14}-adenine and 2-C^{14}-uracil of high specific activities were used in these experiments). It is noteworthy that another yeast enzyme has been purified which catalyzes the condensation of adenine with PRPP to form A5P[9] and which is inactive in forming O5P from orotic acid. O5P decarboxylase also is specific for O5P, attacking neither orotidine nor orotic acid.

(9) A. Kornberg, I. Lieberman and E. S. Simms, unpublished results.

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STRUCTURE OF A NEW AMINO ACID OBTAINED FROM ROSEOTHRICIN[1]

Sir:

Roseothricin (H-277) is a streptothricin-like antibiotic obtained from *Streptomyces roseochromogenus*.[2] Though fairly toxic, it possesses high antibacterial activity. The hydrolysis of roseothricin hydrochloride with 20% hydrochloric acid in a sealed tube for 48 hours at 100° gave two ninhydrin-positive products, which were separated by solubility differences of their picrates. One was β,ε-diaminohexanoic acid, an amino acid also present in streptothricin,[3] streptolin,[3,4] and viomycin,[3,5] and characterized by American workers.[6,7] The other substance, for which the trivial name of "roseonine" is designated, has now been shown to be 2-amino-4(or 5)-(1-carboxy-1-hydroxy-2-amino)-

(1) An imidazoline structure was postulated at the 6th Annual Meeting, Chemical Society of Japan, Kyoto, April, 1953.

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(4) E. E. Smissman, *et al.*, Abstracts of Papers 121st Meeting, American Chemical Society, Milwaukee, Wisconsin, April, 1952, p. 80.

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(7) E. E. van Tamelen and E. E. Smissman, *ibid.*, **74**, 3714 (1952).