phosphate donor such as adenosinetriphosphate.

With the purified phosphohexokinase, K_s values for the various nucleotides have been determined as follows: ATP, 3×10^{-5} *M*; ITP, 7×10^{-5} *M*; and UTP, 3.3×10^{-5} *M*. The maximum velocity is only slightly greater with ATP than with the other nucleotides. ADP does not enhance the rate of phosphorylation in the presence of UTP. These results make it highly unlikely that nucleoside diphosphokinase[10] and ADP participate in the phosphorylation of fructose-6-phosphate by UTP or ITP with the purified muscle phosphohexokinase.

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RECEIVED MARCH 22, 1954

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ALKALOIDS OF *RAUWOLFIA SERPENTINA* BENTH. III.[1] RESCINNAMINE, A NEW HYPOTENSIVE AND SEDATIVE PRINCIPLE

Sir:

The interest in Rauwolfia therapy for the treatment of hypertension has prompted extensive chemical investigations[2] of the Indian plant *Rauwolfia serpentina* Benth in a search for its physiologically active principles. Recently the isolation and structural elucidation of reserpine, an alkaloid possessing pronounced sedative and hypotensive activity, has been reported.[3]

Extensive pharmacological[4] and clinical[4] comparison between reserpine and an alkaloidal extract[5] of *Rauwolfia serpentina* indicated, however, that reserpine could not account for all of the hypotensive and sedative activity of this fraction. As a result of further chemical studies we now wish to report on another highly active alkaloid, **rescinnamine,** the 3,4,5-trimethoxycinnamic acid ester of methyl reserpate.

The isolation of rescinnamine from its natural source was effected by subjecting the benzene soluble portion of the alkaloidal extract,[5] after removal of reserpine by crystallization from methanol, to chromatographic separation on acid washed alumina. An amorphous fraction was obtained which readily crystallized from benzene yielding rescinnamine as fine needles, m.p. 238–239° (vac.), $[\alpha]^{24}D$ -97 ± 2 (*c* 1.0, in $CHCl_3$). Analytical data indicated the empirical formula $C_{35}H_{42}O_9N_2$: Calcd. C, 66.23; H, 6.67; N, 4.41; OCH_3, 29.34; mol. wt., 634.71. Found: C, 66.24; H, 6.62; N, 4.45; OCH_3, 28.81; equiv. wt., 636[6]; pK'_a, 6.4.[6]

On basic hydrolysis with 0.75 *N* sodium hydroxide in methanol–water, rescinnamine yielded 3,4,5-trimethoxycinnamic acid and reserpic acid. The 3,4,5-trimethoxycinnamic acid (m.p. 126.5–127°) gave no depression of melting point on admixture with an authentic sample. The infrared spectra and ultraviolet spectra were identical. Calcd. for $C_{12}H_{14}O_5$: C, 60.50; H, 5.92; OCH_3, 39.08. Found: C, 60.46; H, 5.95; OCH_3, 38.94[7]; methyl ester, m.p. 96.5–97°.[8]

Reserpic acid, isolated as its hydrochloride, was identified by comparison of its hydrochloride and methyl ester derivatives with authentic samples prepared from reserpine.

The infrared spectrum (nujol) of rescinnamine is similar to that of reserpine in the region of the shorter wave lengths (2.5–7 μ) with the exception of a more intense band at 6.19 μ, which may be attributed to the conjugated double bond of 3,4,5-trimethoxycinnamic acid. The ultraviolet spectrum showed λ^{alc}_{max} (log ϵ): 229 mμ (4.73), 302 mμ (4.39); λ^{alc}_{min} (log ϵ): 258 mμ (3.88). The band at 302 mμ is a summation of the α,β-disubstituted 6-methoxyindole and 3,4,5-trimethoxycinnamate chromophores.

Pharmacological tests on rescinnamine show it to have hypotensive, bradycardic and sedative activity similar to that of reserpine. More complete data on these evaluations will be published elsewhere by Dr. G. E. Cronheim.

(1) Papers I and II, THIS JOURNAL, **75**, 4867 (1953); **76**, 1332 (1954).

(2) For a comprehensive review of earlier work see Asima Chatterjee (nee Mookerjee), "Fortschritte der Chemie Organischer Naturstoffe," Vol. 10, Springer-Verlag, Vienna, Austria, 1953, pp. 390–417.

(3) *Cf.* L. Dorfman, A. Furlenmeier, C. F. Huebner, R. Lucas, H. B. MacPhillamy, J. M. Mueller, E. Schlittler, R. Schwyzer and A. F. St. Andre, *Helv. Chim. Acta*, **37**, 59 (1954), and references cited therein.

(4) This work was carried out by the biological sciences and clinical sections of this Laboratory.

(5) This work was carried out on an alkaloidal extract of *Rauwolfia serpentina*, generically designated "alseroxylon," which is available from Riker Laboratories, Inc., Los Angeles, California.

(6) Potentiometric titration in 75% dimethylformamide–water with 0.01 *N* HCl.

(7) Microanalyses by Dr. Adalbert Elek.

(8) H. P. King and Wei-Yuan Huang, THIS JOURNAL, **71**, 1836 (1949).

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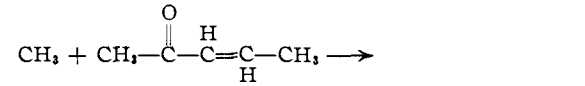
RECEIVED APRIL 1, 1954

A VAPOR-PHASE FREE RADICAL ADDITION-ELIMINATION: REPLACEMENT OF ACETYL BY METHYL[1]

Sir:

We have obtained substantial evidence for a vapor-phase free radical "addition–elimination reaction," (1) in which a methyl radical adds to the olefinic double bond of *trans*-methyl propenyl ketone and an acetyl radical is eliminated. At temperatures above 120° the acetyl radical formed in (1) rapidly dissociates and the following over-all chain sequence is believed to occur



$$CH_3CH{=}CHCH_3 + CH_3CO \quad (1)$$

$$CH_3CO \longrightarrow CH_3 + CO \quad (2)$$

The results of the following experiments can best be explained by assuming the existence of this new type of radical process.[2]

A. Photolyses of *trans*-Methyl Propenyl Ketone at 2380 Å.—2-Butene was found to be the major hydrocarbon product from all direct photolyses of *trans*-methyl propenyl ketone at 2380 Å. and various temperatures, pressures and intensities.

(1) The authors gratefully acknowledge the support of the Atomic Energy Commission through contract AT(11-1)-89, Project No. 4.

(2) For a recent review of reactions of methyl radicals see A. F. Trotman-Dickenson, *Quart. Rev.*, **VII**, 198 (1953).

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 ($\lambda_{280}/\lambda_{260}$ = 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.

(2) L. D. Wright, C. S. Miller, H. R. Skeggs, J. W. Huff, L. L. Weed and D. W. Wilson, THIS JOURNAL, **73**, 1898 (1951).

(3) H. Arvidson, N. A. Eliasson, E. Hammerstein, P. Reichard, H. von Ubich and S. Bergström, *J. Biol. Chem.*, **179**, 169 (1949); R. B. Hurlbert and V. R. Potter, *ibid.*, **195**, 257 (1952).

(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).