gated by the substitution of this analog for DPN or triphosphopyridine nucleotide (TPN) in the specific assay systems for the following pyridine nucleotide-requiring oxidative enzymes[7]: alcohol dehydrogenase (DPN), α-keto-glutaric dehydrogenase (DPN), malic dehydrogenase (DPN), β-hydroxy-acyl CoA dehydrogenase (DPN), isocitric dehydrogenase (TPN) and malic enzyme (TPN). D-INAH-N is not reduced in these reactions. Furthermore, the reduction by their specific dehydrogenases of either DPN or TPN is not inhibited by D-INAH-N. The oxidation of DPNH by the specific DPN-cytochrome *c* reductase[7] of pig heart muscle is not inhibited by D-INAH-N. These several pyridine nucleotide-requiring dehydrogenases were tested in order to obtain as broad a picture as possible of the potential inhibiting nature of this analog.

This anomalous behavior may be interpreted as evidence that DPN is normally reduced by the addition of a proton to the 4-position of the pyridine ring; this position is apparently unavailable in D-INAH-N where the pyridine ring is substituted in the *para* position. This confirms the recent report by Pullman, *et al.*,[8] who investigated the stereospecificity of DPN reduction. These workers concluded from isotope experiments that DPN was reduced in the *para* position of the pyridine ring. The antituberculous action of INAH may accordingly be due to the intracellular formation of an inactive pyridine nucleotide analog with a concomitant reduction in cellular oxidative metabolism.

(7) Generously supplied by Drs. H. R. Mahler, D. R. Sanadi and S. Wakil, Enzyme Institute, University of Wisconsin.

(8) M. E. Pullman, A. San Pietro and S. P. Colowick, *J. Biol. Chem.*, **206**, 129 (1954).

RESEARCH DIVISION
VETERANS ADMINISTRATION HOSPITAL DEXTER S. GOLDMAN
MADISON, WISCONSIN

RECEIVED FEBRUARY 3, 1954

URIDINE- AND INOSINE-TRIPHOSPHATES AS PHOSPHATE DONORS FOR PHOSPHOHEXOKINASE[1]

Sir:

The widespread natural occurrence of the 5'-triphosphates of uridine[2-6] (UTP), inosine[6] (ITP) and other nucleosides raises the question of whether they participate directly in phosphorylating reactions[3,7] in addition to their possible role in nucleic acid synthesis.

Kleinzeller[8] reported ITP to be inactive in the phosphorylation of fructose-6-phosphate with muscle extract as the enzyme source, but Muntz[9] reported this nucleotide to be half as active as ATP when a partially purified phosphofructokinase from brain was employed. The slow rate of glucose phosphorylation by UTP and ITP may, according to Berg and Joklik,[10] result from enzymatic transphosphorylation between these respective nucleotides and ADP, followed by utilization of the formed ATP for the hexokinase reaction. This raises the question whether other cases, where ITP has been found active as a phosphate donor, might have involved a similar transphosphorylation to adenine nucleotide.

A direct utilization of both ITP and UTP for the phosphorylation of fructose-6-phosphate to fructose-1,6-diphosphate has been found to be catalyzed by a highly purified phosphofructokinase (Table I).

TABLE I

PHOSPHORYLATION OF FRUCTOSE-6-PHOSPHATE BY VARIOUS NUCLEOSIDE 5'-TRIPHOSPHATES

The reaction mixture contained 2 μmoles of fructose-6-phosphate, 1 μmole of diphosphopyridine nucleotide, 20 μmoles of cysteine, 10 μmoles of arsenate, 300 μg. of triosephosphate dehydrogenase, 100 μg. of aldolase, 100 μmoles of tris-(hydroxymethyl)-aminomethane HCl buffer (*p*H 8.3), nucleotides as indicated and $MgCl_2$ at a molar concentration equal to that of the nucleotides. Final volume 3.0 ml. The reaction was initiated by addition of 0.2 μg. of phosphofructokinase. The rate of DPN reduction is zero without added nucleotide or without any of the other reaction constituents save cysteine and arsenate. Stock solutions of all enzymes (kept in ammonium sulfate solutions) were diluted with 0.5% serum albumin in 0.01 *M* tris-buffer. ATP and UTP were chromatographed samples from Pabst; ITP obtained from Sigma Chemical Co.

Prof. Herman M. Kalckar kindly supplied a sample of highly purified UTP known to contain less than 0.01% of ATP. It gave similar results.

Phosphate donor (μmoles)	$\Delta \log I_0/I$ at 340 mμ per minute — Minutes 1st	2nd	3rd	4th	5th
ATP, 6	0.035	0.040	0.045	0.047	0.048
UTP, 4.3	.033	.037	.043	.043	.044
UTP, 0.1	.027	.025	.032	.026	.028
UTP, 0.1 + ADP, 0.44	.023	.026	.031	.030	.031
ITP, 3.9	.025	.040	.041	.041	.041
ITP, 0.1[a]	.010	.012	.010	.010	.010

[a] Purified by the method of Cohn and Carter.[11]

The enzyme was extracted from homogenized muscle of magnesium-anesthetized[12] rabbits with 0.03 *M* KOH[13] and was purified by fractionation with alcohol and with ammonium sulfate. The procedure results in a 200-fold purification of the enzyme from the original extract. At this stage of purity 100,000 g. of enzyme phosphorylates about 10,400 moles of fructose-6-phosphate per minute at 26°. It is free from detectable myokinase, triosephosphate isomerase, aldolase, triosephosphate dehydrogenase, α-glycerophosphate dehydrogenase and phosphohexoisomerase. The activity is determined by the rate of reduction of diphosphopyridine nucleotide in a system[14] containing recrystallized aldolase[15] and triosephosphate dehydrogenase,[16] DPN, fructose-6-phosphate, Mg^{++} and a

(1) Supported in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

(2) S. A. Kuby, M.S. Thesis, University of Wisconsin, Madison (1950).

(3) A. Kornberg, *Phosphorus Metabolism*, **1**, 392 (1951).

(4) S. H. Lipton, S. A. Morell, A. Frieden and R. M. Bock, THIS JOURNAL, **75**, 5449 (1953).

(5) R. Bergkvist and A. Deutsch, *Acta Chem. Scand.*, **7**, 1307 (1953).

(6) H. Schmitz, V. R. Potter, R. B. Hurlbert and D. White, *Cancer Research*, **14**, 66 (1954).

(7) M. F. Utter and K. Kurahashi, THIS JOURNAL, **75**, 758 (1953).

(8) A. Kleinzeller, *Biochem. J.*, **36**, 729 (1942).

(9) J. A. Muntz, *Arch. Biochem. Biophys.*, **42**, 435 (1953).

(10) P. Berg and W. K. Joklik, *Nature*, **172**, 1008 (1953).

(11) W. E. Cohn and C. E. Carter, THIS JOURNAL, **72**, 4273 (1950).

(12) K. P. DuBois, H. Albaum and V. R. Potter, *J. Biol. Chem.*, **147**, 699 (1943).

(13) J. F. Taylor, *Federation Proc.*, **6**, 297 (1947).

(14) T. Bücher, *Biochem. Biophys. Acta*, **1**, 292 (1947).

(15) J. F. Taylor, A. A. Green and G. T. Cori, *J. Biol. Chem.*, **173**, 591 (1948).

(16) G. T. Cori, M. W. Slein and C. F. Cori, *ibid.*, **173**, 605 (1948).

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.

INSTITUTE FOR ENZYME RESEARCH
UNIVERSITY OF WISCONSIN — KUO-HUANG LING[17]
MADISON, WISCONSIN — HENRY A. LARDY

RECEIVED MARCH 22, 1954

(17) Postdoctorate Fellow, National Heart Institute.

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).

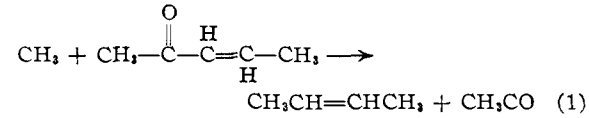
RIKER LABORATORIES, INC. — M. W. KLOHS
8480 BEVERLY BOULEVARD — M. D. DRAPER
LOS ANGELES 48, CALIFORNIA — F. KELLER

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).