H, 8.01.) Treatment of VI with 48% hydriodic acid gave 19-hydroxyprogesterone (VIII), m.p. 171–172°, $[\alpha]^{24}D$ +185°, λ_{max}^{EtOH} 242 mμ, ε 12,900. (*Anal.* Found: C, 75.88; H, 9.11.) Acetylation of VIII produced 19-acetoxyprogesterone (IX), double m.p. 89–95° and 125–126°, $[\alpha]^{25}D$ +212°, λ_{max}^{EtOH} 239 mμ, ε 17,300. (*Anal.* Found: C, 73.45; H, 8.69; loss on drying, 4.26.) All rotations were determined in chloroform.

In bioassays conducted in the laboratory of Dr. John A. Luetscher, Jr., Stanford University School of Medicine, 19-hydroxy-11-desoxycorticosterone (V) produced only a slight sodium-retaining action (approx. 4% that of DOCA). In the Ingle work test, performed by E. H. Morley, W. W. Byrnes and K. J. Olson of the Research Division of the Upjohn Company, V was found to possess less than 2% of the activity of hydrocortisone. 19-Hydroxyprogesterone (VIII) was examined for progestational activity by Dr. Roy Hertz of the National Cancer Institute. Bioassays by the Corner–Allen and Clauberg procedures indicated that VIII is less than 10% as active as progesterone. A detailed report of these findings will appear as "Investigations on Steroids. XXIV" from this laboratory.

DIVISION OF STEROID RESEARCH
THE JOHN HERR MUSSER DEPARTMENT OF RESEARCH MEDICINE
UNIVERSITY OF PENNSYLVANIA G. WINSTON BARBER
PHILADELPHIA 4, PA. MAXIMILIAN EHRENSTEIN

RECEIVED FEBRUARY 26, 1954

ENZYMATIC SYNTHESES OF PYRIMIDINE AND PURINE NUCLEOTIDES.[1] I. FORMATION OF 5-PHOSPHORIBOSYLPYROPHOSPHATE

Sir:

In studies on the incorporation of orotic acid into pyrimidine nucleotides, we have observed its conversion to uracil by liver preparations and a requirement for adenosine triphosphate (ATP) and ribose-5-phosphate (R5P) for this reaction.[2] With an enzyme preparation purified about 20-fold from extracts of pigeon liver acetone powder, the following reaction has been observed

ATP + R5P ⟶ 5-phosphoribosylpyrophosphate (PRPP) + adenosine-5′-phosphate (A5P) (1)

With PRPP isolated from reaction (1) and with a partially purified enzyme preparation from yeast, we have been able to show that adenine is converted to A5P and that uridine-5′-phosphate (U5P) is formed from orotic acid. The evidence, to be presented at a later date, suggests the equations

Adenine + PRPP ⇌ A5P + inorganic pyrophosphate (PP) (2)

Orotic acid + PRPP ⇌ orotidine-5′-phosphate + PP (3a)

Orotidine-5′-phosphate ⟶ U5P + CO_2 (3b)

The further metabolism of U5P leading to the production of uracil and, in the presence of ATP, to the formation of uridine diphosphate (UDP) and uridine triphosphate (UTP) has been demonstrated with enzyme preparations from yeast and liver. These reactions, the mechanisms of which are now under investigation, are summarized in equations (4) and (5).

U5P ⟶ uracil (4)

U5P ⟶ UDP + UTP (5)

Balance studies in support of equation (1) are given in Table I.

TABLE I

STOICHIOMETRY OF PRPP SYNTHESIS[a]

	Micromoles 0 min.	Micromoles 60 min.	Δ
ATP[b]—Exp.	12.5	1.6(1.8)[e]	−10.9
—Control	13.1	12.5(11.1)[e]	− 0.6
PRPP[c]—Exp.	0.0	10.9(10.0)[e]	+10.9
—Control	0.0	0.0	0.0
A5P[d]—Exp.	0.0	9.8(10.2)[e]	+ 9.8
—Control	0.0	0.0(0.0)[e]	0.0

[a] The experimental (Exp.) incubation mixture (10.0 ml.) contained 0.40 ml. of ATP (0.03 *M*, 2.2 × 10^4 c.p.m./μ mole), 1.00 ml. of R5P (0.025 *M*), 0.20 ml. of reduced glutathione (0.5 *M*), 0.20 ml. of $MgCl_2$ (0.1 *M*), 0.50 ml. of KF (1 *M*), 0.20 ml. of phosphate buffer (1 *M*, *p*H 7.4) and 2.00 ml. of the enzyme preparation (containing 0.72 mg. of protein). The control incubation mixture lacked R5P. Incubation was at 35° for 60 min. [b] Determined spectrophotometrically by the combined action of hexokinase and glucose-6-phosphate dehydrogenase (A. Kornberg, *J. Biol. Chem.*, **182**, 779 (1950)). With added myokinase, the extent of TPN reduction was exactly doubled, indicating the absence of adenosine diphosphate (ADP). [c] Determined spectrophotometrically by the removal of orotic acid (see equation (3)), or the production of A5P (see equation (2)); methods are unpublished. [d] Determined spectrophotometrically by Schmidt's A5P deaminase (H. M. Kalckar, *J. Biol. Chem.*, **167**, 445 (1947)). [e] Values in parentheses were determined by chromatography on Dowex-1 anion exchange resin; ATP and A5P were estimated by optical density measurement at 260 mμ; PRPP was estimated as indicated in footnote (*c*).

ATP labeled with P^{32} in the two terminal phosphate groups was used. In the control sample (R5P absent), ATP was not removed to any significant extent and the production of PRPP and A5P was not detectable. In the presence of R5P, almost all the ATP added disappeared and was matched by the appearance of equivalent molar quantities of PRPP and A5P. No ADP or inorganic orthophosphate was produced from ATP after 30 min. or 60 min., when 65 or 87%, respectively, of the ATP was consumed.

PRPP was isolated by ion-exchange chromatography as a discrete symmetrical zone and estimated spectrophotometrically by enzymatic condensation with adenine (equation (2)), or with orotic acid (equation (3)). Eight fractions selected from this PRPP zone (representing approximately 80% of the PRPP) contained pentose, enzymatically active PRPP (equations 2 or 3), acid-labile P and total P in molar ratios (within 5% of the average value) of 1.00:0.94:2.04:3.08. The average specific radioactivity (c.p.m./μmole) of these fractions was 2.24 × 10^4 as compared with values of 2.27 × 10^4 and 2.14 × 10^4, respectively, for the

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

(2) I. Lieberman, A. Kornberg, E. S. Simms, and S. R. Kornberg, *Federation Proc.*, in press. B. Karger and C. E. Carter have also obtained evidence for the conversion of orotic acid to uracil by liver extracts with uridylic acid as an intermediate (personal communication).

ATP at zero time and that isolated from the control sample.

A solution of PRPP, containing 1.45 μmoles of enzymatically active material was heated at 65° in 0.1 *M* acetate buffer, *p*H 4.0. After 10 and 40 minutes, respectively, 0.51 and 0.00 μmole of enzymatically active PRPP remained; 0.96 and 1.73 μmoles of reducing substance (referred to ribose) appeared. In another experiment involving a 30 min. heating period, the removal of 1.13 μmoles of PRPP was matched by the formation of 1.07 μmoles of reducing substance and 0.99 μmole of PP (determined by ion-exchange analysis); 0.30 μmole of inorganic orthophosphate was also produced.

These observations taken together with evidence, to be presented later, for the quantitative conversion of PRPP to A5P (equation (2)) or to U5P (equation (3)) lead us to propose a provisional structure of 5-phosphoribosylpyrophosphate for the activated ribose compound.

ADP did not replace ATP in equation (1) and ribose-1-phosphate (R1P) reacted at only 11% of the rate observed with R5P; this reactivity of R1P is likely due to its conversion to R5P by phosphoribomutase activity in the enzyme preparation. A sample presumed to contain ribose-1,5-diphosphate,[3] derived from glucose-1,6-diphosphate[4] and R1P by the action of phosphoglucomutase[5] and glucose-6-phosphate dehydrogenase, was inactive in place of PRPP in equation (3).

It is evident that PRPP may also prove to be the intermediate involved in the synthesis of ribotides of acylic purine precursors,[6] nicotinamide[7] and other nitrogenous compounds, and in the system for A5P synthesis described by Saffran and Scarano.[8] There is the further possibility that a 2-deoxyPRPP will prove to be the active condensing agent in the biosynthesis of deoxynucleotides.

(3) H. Klenow, *Arch. Biochem.*, **46**, 186 (1953).
(4) Kindly furnished by Dr. L. F. Leloir.
(5) Kindly furnished by Dr. D. H. Brown.
(6) G. R. Greenberg, *J. Biol. Chem.*, **190**, 611 (1951); W. J. Williams and J. M. Buchanan, *ibid.*, **203**, 583 (1953).
(7) I. G. Leder and P. Handler, *ibid.*, **189**, 889 (1951).
(8) M. Saffran and E. Scarano, *Nature*, **174**, 949 (1953).

DEPARTMENT OF MICROBIOLOGY
WASHINGTON UNIVERSITY
SCHOOL OF MEDICINE
ST. LOUIS 10, MO.

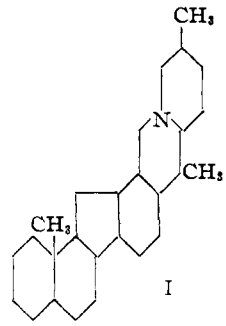
ARTHUR KORNBERG
IRVING LIEBERMAN
ERNEST S. SIMMS

RECEIVED MARCH 5, 1954

THE VERATRINE ALKALOIDS. XXXVIII. THE RING SYSTEM OF THE TERTIARY POLYHYDROXY VERATRINE BASES. OXIDATIVE STUDIES WITH CEVANTHRIDINE AND VERANTHRIDINE

Sir:

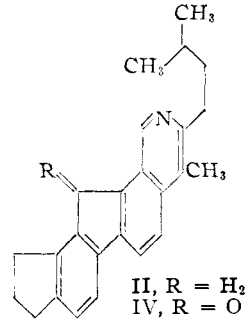
In a recent paper, a ring system (I) was suggested for veracevine,[1] cevine, germine and protoverine which was based on the chemical behavior and absorption spectra of several selenium dehydrogenation products of cevine.[2] The two largest basic fragments, cevanthridine,[3] $C_{25}H_{27}N$, and a



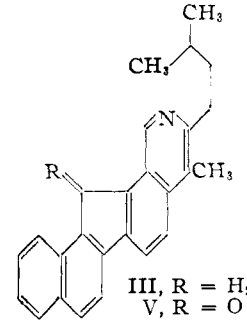
I

$C_{26}H_{25}N$ base[4] now named *veranthridine* were formulated as 1,2-cyclopentenofluorene and 1,2-benzofluorene derivatives, respectively, (II) and (III). We now wish to report a series of experiments with these degradation products which lend support to our earlier conclusions.

Oxidation of cevanthridine (II) with chromium trioxide in acetic acid (75°, ten minutes) gave a complex mixture from which a ketobase (IV) (titrates against perchloric acid in acetic acid) was separated by chromatography over alumina. Crystallization from chloroform gave brilliant reddish-orange needles, m.p. 253–255° cor. Calcd. for $C_{25}H_{25}NO$: C, 84.47; H, 7.09. Found: C, 84.47; H, 7.12. ($\lambda_{max.}$: 277 mμ, log ε 4.90; 366 mμ, log ε 4.15 (EtOH). IV was also formed in almost quantitative yield by simply shaking a solution of II in hot ethanolic sodium ethoxide in air. Huang-Minlon reduction of IV regenerated cevanthridine, m.p. 211.5–213.5° cor., undepressed when admixed with an authentic specimen. Calcd. for $C_{25}H_{27}N$: C, 87.93; H, 7.97. Found: C, 87.91; H, 7.83. The ultraviolet spectrum was indistinguishable from that of authentic cevanthridine. A similar oxidation of veranthridine (III) proceeded smoothly to give a good yield of the corresponding ketobase



II, R = H₂
IV, R = O



III, R = H₂
V, R = O

(V) (titrates against perchloric acid in acetic acid), reddish-orange needles, m.p. 267–270° cor. Calcd. for $C_{26}H_{23}NO$: C, 85.45; H, 6.34. Found: C, 85.66; H, 6.34 ($\lambda_{max.}$ 265 mμ, log ε 4.60; 302 mμ, log ε 4.89; 342 mμ, log ε 4.08). This same substance was also isolated from the basic fraction of the selenium dehydrogenation mixture from cevine by chromatography over alumina; m.p. 266–269°. Calcd. for $C_{26}H_{23}NO$: C, 85.45; H, 6.34; N, 3.83. Found: C, 85.37, 85.41; H, 6.37, 6.21; N, 3.82. The ultraviolet spectrum was identical with that of V prepared by chromic acid oxidation. The occurrence of V among the dehydrogenation products of cevine is due presumably to its ready formation

(1) S. W. Pelletier and W. A. Jacobs, THIS JOURNAL, **75**, 3248 (1953); S. M. Kupchan and D. Lavie, *ibid.*, **76**, 314 (1954).
(2) W. A. Jacobs and S. W. Pelletier, *J. Org. Chem.*, **18**, 765 (1953).
(3) B. K. Blount, *J. Chem. Soc.*, 122 (1935); 414 (1936); L. C. Craig and W. A. Jacobs, *J. Biol. Chem.*, **139**, 293 (1941).
(4) L. C. Craig and W. A. Jacobs, *ibid.*, **139**, 263 (1941).