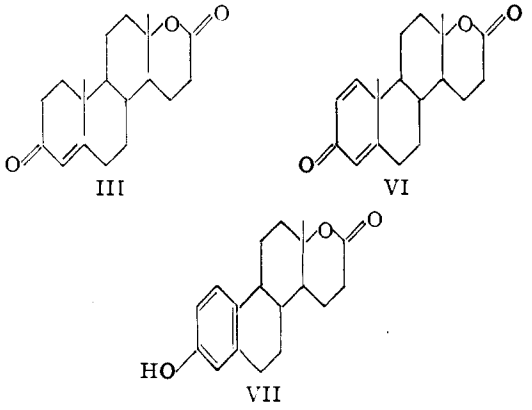
acteristic of $\Delta^{1,4}$-3-semicarbazones.[4] Pyrolysis of VI at 550–600° in mineral oil[11] furnished Westerfeld's lactone (estrololactone)[12,13] (VII), identified after conversion into the acetate, m.p. 149–151°; $\lambda_{max.}^{alc.}$ 267 mμ(ϵ = 1080) and 275 mμ(ϵ = 950); $\lambda_{max.}^{Nujol}$ 5.70 μ (phenolic acetyl), 5.80 μ (lactone carbonyl), by comparison with an authentic sample of the latter.[8]



III VI VII

The biochemical conversion of the β-oriented acetyl side chain in progesterone to a 17β-hydroxyl group parallels the degradation of 20-ketosteroids by peracids, which likewise proceeds with retention of configuration at C_{17}.[14] Similarly, the formation of ring D lactones has its chemical parallel in the reaction sequence: progesterone $\xrightarrow{RCO_3H}$ testosterone $\xrightarrow{-H_2}$ androstenedione $\xrightarrow{RCO_3H}$ III. A bioöxidation mechanism involving these same intermediates is not out of the question since testosterone is readily converted into VI by *C. radicola.*

(11) E. B. Hershberg, M. Rubin and E. Schwenk, *J. Org. Chem.*, **15**, 232 (1950).

(12) W. W. Westerfeld, *J. Biol. Chem.*, **143**, 177 (1943).

(13) R. P. Jacobsen, *ibid.*, **171**, 61 (1947).

(14) T. F. Gallagher and T. Kritchevsky, THIS JOURNAL, **72**, 882 (1950); R. B. Turner, *ibid.*, **72**, 878 (1950).

THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH
NEW BRUNSWICK, NEW JERSEY

JOSEF FRIED
RICHARD W. THOMA
ANNA KLINGSBERG

RECEIVED OCTOBER 19, 1953

THE SYNTHESIS OF ERYTHRINANE[1]

Sir:

The purpose of this communication is to report on a simple method of probable general applicability for the elaboration of the entire ring system of the *Erythrina abyssinica* Lam. alkaloids, the constitution of which has been elucidated recently by Prelog and his co-workers.[2] The conversion of 2-carbethoxycyclohexanone to erythrinane in five steps and about 12% over-all yield is described. It is anticipated that a number of substituted erythrinanes will now be easily accessible for chemical and pharmacological studies.

(1) The term "erythrinane" is proposed to designate the basic ring system of the Erythrina alkaloids.

(2) M. Carmack, B. C. McKusick and V. Prelog, *Helv. Chim. Acta*, **34**, 1601 (1951); H. G. Khorana, G. W. Kenner and V. Prelog, *ibid.*, **34**, 1969 (1951). Concerning the structure of β-erythroidine, the exhaustive investigations of Boekelheide and his collaborators (ref. 7 and accompanying papers) should be consulted.

Through the condensation of the potassio derivative of 2-carbethoxycyclohexanone with 2-bromoethylphthalimide in boiling toluene, there was obtained as a viscous oil, 2-carbethoxy-2-(β-phthalimidoethyl)-cyclohexanone which, without purification, was hydrolyzed with boiling concentrated hydrochloric acid to 2,3,4,5,6,7-hexahydroindole (35% over-all yield), [b.p. 80° (19 mm.)]; *Anal.* Calcd. for $C_8H_{13}N$: N, 11.37. Found: N, 11.28. *Picrate,*[3] m.p. 132–133° [*Anal.* Calcd. for $C_{14}H_{16}O_7N$: C, 47.72; H, 4.57. Found: C, 47.85; H, 4.45]. Over Adams catalyst in ethanol, the latter base absorbed 0.98 molar proportion of hydrogen to yield octahydroindole isolated as the picrate, m.p. 135–137°, which proved identical with a sample prepared according to the literature.[4] Treatment of hexahydroindole with phenylacetyl chloride under the Schotten-Baumann conditions afforded in 80% yield, *2-(β-phenylacetamidoethyl)-cyclohexanone* (I), m.p. 53–54°, (*Anal.* Calcd. for $C_{16}H_{21}O_2N$: C, 74.09; H, 8.16. Found: C, 73.96; H, 8.18.); maxima at 2.92 μ (NH—), 5.85 μ (C=O), 6.02 and 6.60 μ (—CONH—) and 6.70 μ (phenyl) in the infrared region. When heated for twenty-four hours at 100° in excess polyphosphoric acid, (I) was converted in 60% yield to *8-oxoerythrinane*[5] (II), m.p. 132–133° (*Anal.* Calcd. for $C_{16}H_{19}ON$: C, 79.62; H, 7.93; N, 5.80. Found: C, 79.71; H, 8.02; N, 5.62.), whose structure was deduced from the following evidence: (a) its infrared spectrum shows a single peak at 6.14 μ (besides the phenyl bands) which is characteristic of a disubstituted amide carbonyl group; (b) it is unaffected by prolonged heating in concentrated hydrochloric acid, a behavior inconsistent with an acyclic amide structure but consistent with a lactam structure[6]; (c) when treated with lithium aluminium hydride in boiling ether it is converted in 70% yield to the corresponding base *erythrinane,* a colorless oil, b.p. 195° (bath temp.) (0.1 mm.) (*Anal.* Calcd. for $C_{16}H_{21}N$: C, 84.52; H, 9.31. Found: C, 84.38; H, 9.17.); *picrate:* m.p. 184–185° (*Anal.* Calcd. for $C_{22}H_{24}O_7N_4$: N, 12.27. Found: N, 12.29); *methiodide:* m.p. 201–203° (*Anal.* Calcd. for $C_{17}H_{24}NI$: I, 34.3. Found: I, 34.1.). The infrared spectrum of the free base lacks the band of (II) at 6.14 μ; (d) on vigorous oxidation with nitric acid it yields 4-nitrophthalic acid isolated as its anhydride and further characterized as its anil derivative; both proved identical with authentic specimens.

Therefore, (II) is the only reasonable structure accommodating the evidence and further work in the methoxylated series is contemplated.

The biogenetic implications of the facile conversion of (I) to (II) are obvious and in keeping with Boekelheide's recent suggestion.[7]

Several attempts to ring close N-(β-phenylethyl)-

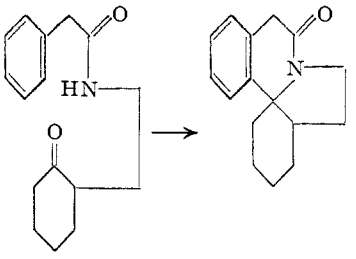
(3) Boiling points and melting points are uncorrected.

(4) R. Willstätter and D. Jaquet, *Ber.*, **51**, 767 (1918), report 137–138° as the m.p. of octahydroindole picrate.

(5) For the numbering of the ring system, see ref. 2.

(6) The opened form of the lactam undoubtedly exists in the hot acid mixture but cannot be isolated presumably because of spontaneous ring closure during the process of isolation. A similar apparent refractoriness of a lactam to boiling hydriodic acid can be found in the morphinane series (M. Gates, R. B. Woodward, W. F. Newhall and R. Kunzli, THIS JOURNAL, **72**, 1141 (1950)).

(7) V. Boekelheide, *et al.*, *ibid.*, **75**, 2550 (1953).



(I) (II)

hexahydroindole [b.p. 123–124° (0.4 mm.); *Anal.* Calcd. for $C_{16}H_{21}N$: C, 84.52; H, 9.31. Found: C, 84.33; H, 9.45; oily salts; obtained by treating β-phenylethyl bromide with hexahydroindole] to erythrinane, led only to unchanged starting material.

DEPARTMENT OF BIOCHEMISTRY
SCHOOL OF MEDICINE B. BELLEAU[8]
LAVAL UNIVERSITY, QUEBEC

RECEIVED OCTOBER 22, 1953

(8) Reed and Carnrick Co., 155 van Wagener Ave., Jersey City 6, N. J.

ENZYMATIC REDUCTION OF β-ASPARTYL PHOSPHATE TO HOMOSERINE

Sir:

The enzymatic formation of β-aspartyl phosphate from L-aspartate and ATP[1] recently has been reported.[2] A synthetic preparation of this substance now has been obtained, and found to be enzymatically reduced to homoserine in a TPN-dependent system obtained from yeast. The phosphorylation and subsequent reduction of the β-carboxyl group of aspartate to a hydroxyl group appear, in view of recent isotope and genetic work, to represent intermediate steps in the biosynthesis of threonine. The latter was demonstrated by such experiments to arise from aspartate via homoserine.[3,4]

The starting material for BAP[1] synthesis was carbobenzoxy-L-aspartyl-α-benzyl ester β-chloride.[5] To replace the chloride with phosphate this compound was shaken in ether with monosilver phosphate,[6] and the carbobenzoxy and benzyl groups were then removed by hydrogenation over palladium black in cold potassium bicarbonate solution. Because of its extreme lability the product was not further purified. It was characterized by the β-asparthydroxamic acid, identified chromatographically, formed on reacting with hydroxylamine. Enzymatic identification was made by its ability to transfer phosphate to ADP.[2] This substance is approximately 30% hydrolyzed in 30 minutes at 30° in aqueous solutions from *p*H 4 to 10. In such solutions at 15° it is relatively stable for several hours.

The enzyme preparation used in these experiments was an extract of baker's yeast partially purified by heating to 55°, discarding the precipitate, and dialyzing 15 hours against cold potassium bicarbonate buffer.

Table I shows the equivalent disappearance of BAP and formation of homoserine in the complete enzyme system. No homoserine appears when the BAP or enzyme is destroyed by brief heating prior to addition. On prolonged incubation complete transformation of BAP to homoserine occurs.

TPN and glutamate markedly increase the extent of the reduction, presumably through the TPN-linked yeast glutamic dehydrogenase[7] which is very active in the protein preparation. Glutamate may be replaced by glucose plus the glucose dehydrogenase of ox liver.[8] Though the latter reduces both DPN and TPN, only TPN serves to reduce BAP in its presence.

TABLE I

Homoserine was qualitatively identified by paper chromatography in water-saturated phenol with 0.3% NH_3, and in the two alternate solvent systems recommended by Redfield.[9] It was further identified by conversion to its DNP-derivative,[10] which was chromatographed on paper.[11] Quantitative estimation of homoserine was made by eluting the DNP-homoserine from paper chromatograms and analyzing spectrophotometrically at 362 mμ. BAP was determined as acyl phosphate.[12] The complete system contained in 1.0 ml. 100.0 μM. TEA-chloride buffer, 0.16 μM. TPN (63% pure), 5.0 μM. TEA-glutamate, 2.7 μM. BAP, and 0.27 ml. of enzyme. The *p*H was 7.9 and the incubation was at 15° for 60 minutes.

Experiment	BAP utilized, μM.	Homoserine formed, μM.
Complete system	1.4	1.4
Omit TPN	0.4	0.4
Omit glutamate	0.5	0.5
Heated enzyme used (100°, 5 min.)	0.0	−0.1
Heated BAP used (100°, 10 min., neutral solution)	...	0.1

The enzyme preparation used here causes a rapid splitting of BAP in the presence of potassium arsenate, a point of similarity with 3-phosphoglyceraldehyde dehydrogenase which also catalyzes a reduction and an arsenolysis of an acyl phosphate.[13,14] Both arsenolysis and reduction are inhibited by iodoacetate.

We are grateful to Drs. J. P. Greenstein and A. Meister for gifts of several compounds, and for assistance during the synthesis of BAP.

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
NATIONAL INSTITUTES OF HEALTH SIMON BLACK
PUBLIC HEALTH SERVICE NANCY G. WRIGHT
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
BETHESDA, MD.

RECEIVED OCTOBER 17, 1953

(1) Abbreviations used are BAP (β-aspartyl phosphate), ADP (adenosine diphosphate), ATP (adenosine triphosphate), TPN (triphosphopyridine nucleotide), DPN (diphosphopyridine nucleotide), DNP (dinitrophenyl), and TEA (triethanolamine).

(2) S. Black and N. M. Gray, THIS JOURNAL, **75**, 2271 (1953).

(3) A. M. Delluva, *Arch. Biochem. Biophys.*, **45**, 443 (1953).

(4) M. L. Hirsch and G. N. Cohen, *Compt. rend.*, **236**, 2338 (1953).

(5) M. Bergmann, L. Zervas and L. Salzmann, *Ber.*, **66B**, 1288 (1933).

(6) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **153**, 571 (1944). Excess H_3PO_4 greatly enhances solubility. A mixture of 1 g. Ag_3PO_4 + 0.5 ml. 85% H_3PO_4 was used.

(7) E. Adler, G. Gunther and J. E. Everett, *Z. physiol. Chem.*, **255**, 27 (1938).

(8) H. J. Strecker and S. Korkes, *J. Biol. Chem.*, **196**, 769 (1952). Use of this enzyme was suggested by Dr. E. R. Stadtman. A preparation was supplied by Dr. B. L. Horecker.

(9) R. R. Redfield, *Biochim. et Biophys. Acta*, **10**, 344 (1953).

(10) The conversion of amino acids to DNP-derivatives was by an unpublished method used in Dr. O. H. Lowry's laboratory and communicated to us by Dr. J. L. Strominger.

(11) K. R. Rao and H. A. Sober, submitted for publication. We thank the authors for making information on the chromatography of DNP-homoserine available to us prior to publication.

(12) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 21 (1945).

(13) J. Harting and S. Velick, *Federation Proc.*, **11**, 226 (1952).

(14) E. Racker and I. Krimsky, *J. Biol. Chem.*, **198**, 731 (1952).