# VITAMINS AND HORMONES

## ADVANCES IN RESEARCH AND APPLICATIONS

*Edited by*

ROBERT S. HARRIS *and* KENNETH V. THIMANN

Professor of Biochemistry of Nutrition
Massachusetts Institute of Technology, Cambridge, Mass.

Professor of Plant Physiology,
Harvard University,
Cambridge, Mass.

VOLUME V

1947

## CONTRIBUTORS TO VOLUME V


H. R. Bird, *Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville, Maryland*

Anne C. Carter, *Department of Medicine, Cornell University Medical College and the New York Hospital, New York, New York*

Harry A. Charipper, *Department of Biology, Washington Square College of Arts and Science, New York University, New York, New York*

Eugene J. Cohen, *Department of Medicine, Cornell University Medical College and the New York Hospital, New York, New York*

William J. Darby, *Department of Medicine and Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee*

C. W. Emmens, *National Institute for Medical Research, Hampstead, London, England*

Herbert M. Evans, *Institute of Experimental Biology, University of California, Berkeley, California*

Albert S. Gordon, *Department of Biology, Washington Square College of Arts and Science, New York University, New York, New York*

Carl G. Heller, *Departments of Physiology and Medicine, University of Oregon Medical School, Portland, Oregon*

Choh Hao Li, *Institute of Experimental Biology, University of California, Berkeley, California*

William O. Maddock, *Departments of Physiology and Medicine, University of Oregon Medical School, Portland, Oregon*

Daniel Melnick, *Chief of the Food Development Division, Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois*

Nicholas A. Milas, *Massachusetts Institute of Technology, Cambridge, Massachusetts*

Harold P. Morris, *National Cancer Institute, National Institute of Health, Bethesda, Maryland*

Alexander von Muralt, *Halleranium, Berne, Switzerland*

Bernard L. Oser, *Food Research Laboratories, Inc., Long Island City, New York*

A. S. Parkes, *National Institute for Medical Research, Hampstead, London, England*

Ephraim Shorr, *Department of Medicine, Cornell University Medical College and the New York Hospital, New York, New York*

## Editors' Preface

Articles concerned with the chemical, biological, and clinical properties of vitamins and hormones are appearing in ever-increasing numbers in the scientific journals of the world. In fact, it is now humanly impossible for one to keep abreast with all the advances in knowledge in these fields by reading the original articles. Scientists and clinicians must rely upon critical reviews to keep them informed of advances in fields related to their special interests.

Previous volumes of *Vitamins and Hormones* have been very favorably received. The Editors interpret this as evidence that this publication is fulfilling a need for critical reviews of the field. Suggestions and criticisms will always be welcome. An index to the contents of the five volumes now published is included at the end of this volume for reference.

Volume V was written and published during a period of post-war turbulence. The Editors wish to express their gratitude to the authors whose concentration and devotion under difficult circumstances have led to the publication of the excellent reviews in this volume.

While this preface is not, in general, the place for comments on the literature, the Editors would like to mention the following publication, of which notice has recently been received. "Register der Welt Literatur über Vitamine und der von ihnen beeinfluszten Gebiete" by Dr. M. Stechow, with a preface by Dr. A. Scheunert, is published by Helingsche Verlagsanstalt, Leipzig. The first volume of this massive tabulation of the world's vitamin literature covers the period 1890–1929, and the second volume has been promised for later years.

KENNETH V. THIMANN
ROBERT S. HARRIS

October, 1947

# CONTENTS

**Thiamine and Peripheral Neurophysiology**

By Alexander von Muralt, *Halleranium, Berne, Switzerland*



**The Physiological Effects of the Pteroylglutamates in Man—with Particular Reference to Pteroylglutamic Acid (PGA)**

By William J. Darby, *Department of Medicine and Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee*

**The Vitamin Requirements of Chicks**

By H. R. Bird, *Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville, Maryland*



**Vitamin Requirements of the Mouse**

By Harold P. Morris, *National Cancer Institute, National Institute of Health, Bethesda, Maryland*

**The Properties of the Growth and Adrenocorticotropic Hormones**

By Choh Hao Li and Herbert M. Evans, *Institute of Experimental Biology, University of California, Berkeley, California*

Page

## Effect of Exogenous Estrogens on the Male Mammal

By C. W. Emmens and A. S. Parkes, *National Institute for Medical Research, Hampstead, London, England*



## The Biology of Antithyroid Agents

By Harry A. Charipper and Albert S. Gordon, *Department of Biology, Washington Square College of Arts and Science, New York University, New York, New York*

**The Use of Androgens in Women**

By Anne C. Carter, Eugene J. Cohen and Ephraim Shorr, *Department of Medicine, Cornell University Medical College and the New York Hospital, New York, New York*

**The Clinical Uses of Testosterone in the Male**

By Carl G. Heller and William O. Maddock, *Departments of Physiology and Medicine, University of Oregon Medical School, Portland, Oregon*

# The Synthesis of Vitamin A and Related Products

By NICHOLAS A. MILAS

CONTENTS



## I. Introduction

Since the elucidation of the structure of vitamin A by Karrer, Morf and Schöpp (1931), Karrer and Morf (1933), Heilbron, Heslop *et al.* (1932) and Heilbron, Morton *et al.* (1932) and by others, several synthetic approaches to the vitamin itself or its derivatives have been published. These have been ably reviewed elsewhere by Bogert (1938), by Sobotka and Bloch (1944) and by Heilbron, Jones and Bacharach (1944).

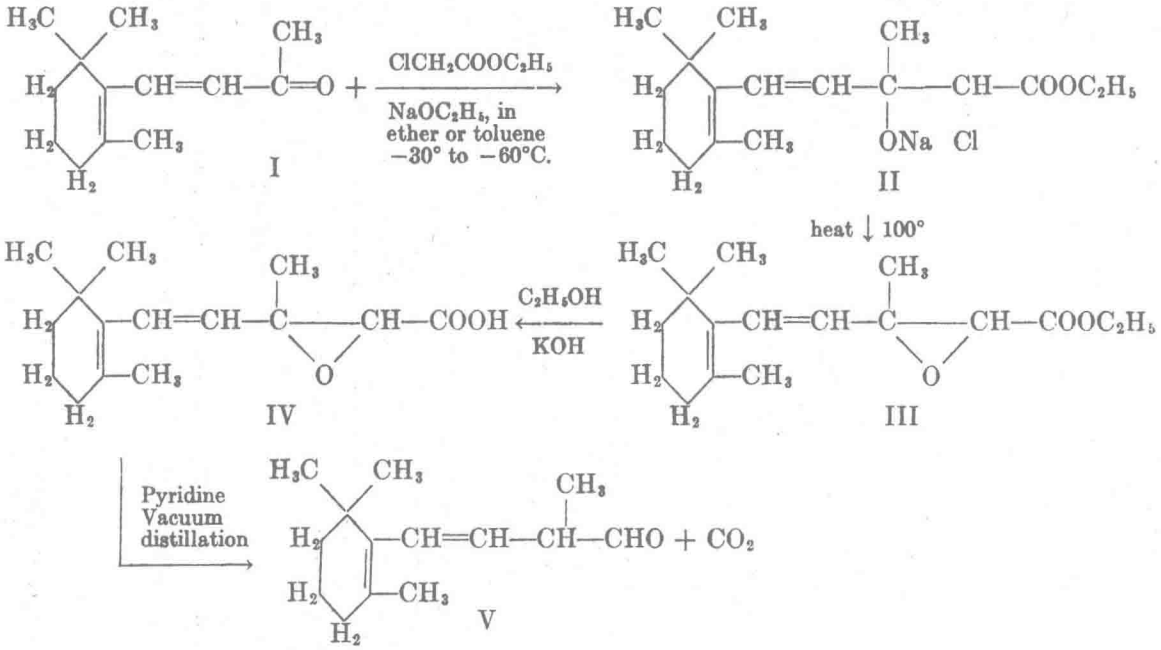
The first claim to a synthesis of vitamin A was made by Fuson and Christ (1936). By allowing β-cyclocitral to condense with β-methylcrotonaldehyde and reducing the resulting product with aluminum isopropoxide, they obtained an alcohol which was assumed to be vitamin A. Spectroscopic evidence was the only proof given to support this claim, which was later disputed by Heilbron and Jones (1936) and by Kuhn and Morris (1937) on the grounds that β-cyclocitral fails to condense with β-methylcrotonaldehyde and the product of Fuson and Christ was merely an open chain polyene aldehyde formed by the autocondensation of β-methylcrotonaldehyde.

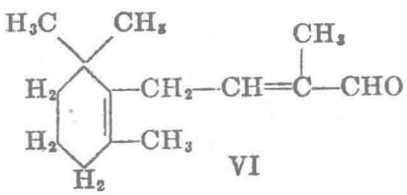
Following along similar lines, Kuhn and Morris (1937; 1940; 1941a,b) published a synthesis of vitamin A in which β-ionylideneacetaldehyde, instead of β-cyclocitral, was condensed with β-methylcrotonaldehyde in the presence of piperidine acetate as a catalyst, and the resulting product, without purification, reduced with aluminum isopropoxide. The mixture obtained was then chromatographed on alumina from which was eluted a specimen having an absorption maximum with antimony trichloride in chloroform of 606 mμ and a biological activity of about 7.5% of the purest vitamin A sample then known. No other analytical data or yields were given by the authors. In view of the importance of vitamin

A during the war, considerable time was spent by several investigators (Sobotka and Bloch, 1944; Heilbron, Jones and Bacharach, 1944; Karrer and Rüegger, 1940; Krauze and Slobodin, 1940) in an effort to repeat this synthesis. In no case was a biologically active product obtained. This synthesis was also investigated in our Laboratory (Milas and Kovitz, 1947), but our failure to obtain β-ionylideneacetaldehyde forced us to abandon it early in 1940. β-Ionylideneacetaldehyde, the key intermediate in the Kuhn-Morris synthesis, has not yet been obtained in the pure state. The original claim of Davies, Heilbron *et al.* (1935) that this aldehyde can be prepared by heating *in vacuo* a mixture of barium β-ionylideneacetate and barium formate has been disputed by Sobotka, Bloch and Glick (1943) who obtained α-ionone and no aldehyde, while, in a more recent study of the same reaction, Karrer and Rüegger (1944) reported the formation of β-ionone. Instead of the barium salts, we heated, under greatly reduced pressure, the corresponding thorium salts and obtained no product possessing aldehydic properties (Milas and Sakal, 1947). A 64% yield of β-ionylideneacetaldehyde has been reported by Krauze and Slobodin (1940) from a reaction of β-ionone with bromoacetal and magnesium. This synthesis was also repeated in this laboratory but no β-ionylideneacetaldehyde was obtained, instead β-ionone was recovered unchanged (Milas and Edgerton, 1947).

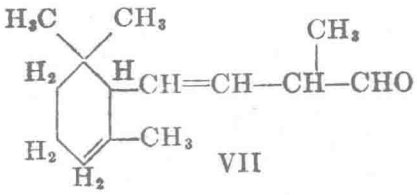
Following a somewhat different scheme (Milas and McAlevy, 1935), Kipping and Wild (1939) published a synthesis of vitamin A methyl ether in which β-ionone was condensed with 1-bromo-4-methyl-6-methoxyhexadiene-2,4 using lithium as the condensing agent and the resulting product dehydrated. Since no experimental details, analyses or biological results were given, it is difficult to evaluate this synthesis.

Early in 1940, the present author (Milas, 1945a–o; Milas, 1946) with several of his associates launched an exhaustive study of several routes, other than those mentioned above, for the synthesis of vitamin A, its derivatives, and its homologues. The key intermediate of several of these syntheses, which ultimately led to the preparation of biologically active vitamin A products, was prepared by the application of the Darzens' synthesis to β-ionone (Milas, 1945a–o; Milas, 1946; Ishikawa and Matsuura, 1937; Heilbron, Johnson *et al.* 1942; Cymerman *et al.* 1946; Milas, Lee, Sakal *et al.* 1947). When β-ionone was condensed with ethyl chloroacetate at low temperatures (−30° to −60°C.) in anhydrous ether or toluene, using alcohol-free sodium ethoxide or methoxide as condensing agents, the glycidic ester (III) was produced, which, upon hydrolysis, gave the glycidic acid (IV). This acid has been decarboxylated by various methods, but the best results were obtained when its pyridine salt was distilled under reduced pressure.


ClCH2COOC2H5
NaOC2H5, in ether or toluene
−30° to −60°C.
I
II
heat ↓ 100°
C2H5OH
KOH
IV
III
Pyridine
Vacuum
distillation
V
+ CO2



VI



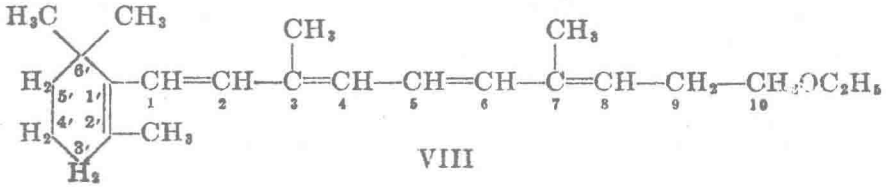
VII

The aldehyde formed by the decarboxylation of the glycidic acid (IV) was extensively investigated by Milas, Lee *et al.* (1947), who demonstrated that it probably has structure (V) rather than structure (VI) proposed by Heilbron, Johnson *et al.* (1942). Ozonization experiments, for example, led to the isolation of geronic acid rather than 3,3-dimethyloctanedione-2,7 which was expected to result from an aldehyde having structure (VI). The formation of small amounts of the isomeric aldehyde (VII) cannot be entirely avoided in spite of the high purity of the $\beta$-ionone used. This is not at all surprising in view of the recent work of Köster (1944) who reported that when pure $\beta$-ionone was treated with sodium alcoholate or with alcoholic alkali, it was partially converted into $\alpha$-ionone. However, when pure $\alpha$-ionone was treated with the same reagents, it was chiefly converted into $\beta$-ionone.

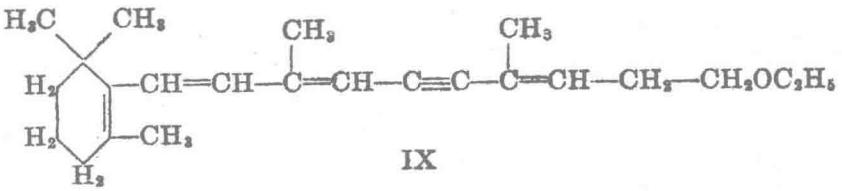
## II. Synthesis of Biologically Active Homologues of Vitamin A Ethers

Two of the earliest biologically active products to be synthesized using the aldehyde (V) as the key intermediate, were [1-(2′,6′,6′-trimethyl-cyclohexen-1′-yl)-3,7-dimeth yldeca-1,3,5,7-tetraenyl]-10-ethyl ether (VIII) or simply homovitamin A ethyl ether, and [1-(2′,6′,6′-trimethylcyclohexen-1′-yl)3,7-dimethyldeca-1,3,7-trien-5-ynyl]-10-ethyl ether or simply 5-dehydrohomovitamin A ethyl ether (IX). These two products have been synthesized by a number of routes (Milas, 1945d; Milas, Lee *et al.* 1947).

In the first step of the synthesis of the 5-dehydrohomovitamin A ethyl ether, the aldehyde (V) was allowed to react in anhydrous ether



VIII



IX

with the Grignard of either 3-methyl-6-ethoxyhexa-3-en-yne-1 (X) or that of 3-methyl-6-ethoxyhexa-1-yn-3-ol (XI). In the first case the carbinol (XII) was formed, while in the second case the glycol (XIII) resulted. Both the carbinol and the glycol were dehydrated in toluene using catalytic amounts of *p*-toluenesulfonic acid to give *inter alia* 5-dehydrohomovitamin A ethyl ether. The crude sample exhibited two bands in the ultraviolet; a broad band at 316–320 mμ and another at 285–290 mμ. It was distilled once under high vacuum and the distillate tested biologically by Professor Robert S. Harris of the Nutritional Laboratories of this Institute. He reported that, when fed to vitamin A-deficient rats in doses of 96γ per day, it cured xerophthalmia and caused an average weight increase per rat of 32 g. for the 28-day test period (see Table III).

Further purification of the 5-dehydrohomovitamin A ethyl ether obtained from the dehydration of the carbinol (XII) was effected first by high vacuum distillation followed by partition between equal volumes of petroleum ether and 90% methanol, then by chromatographic adsorption of the petroleum ether-soluble portion on activated alumina. The unadsorbed portion (main product) was fractionated and the main fraction (light orange oil) boiling at 100–104°C. ($10^{-5}$ mm.) collected and analyzed. It was found to have a single absorption band with a maximum at 321 mμ (Fig. 1, curve A) and to give a purplish-blue color with antimony trichloride in chloroform with absorption maxima at 622 and 580 mμ, respectively. Other analytical data such as hydrogenation and carbon and hydrogen analysis were in accord with formula (IX). The eluate showed two sets of bands very much like those of the original crude product.

The 5-dehydrohomovitamin A ethyl ether obtained from the dehydration of the glycol (XIII) was one of the earliest investigations of the authors and the product was purified in the same manner as in the above case, except that, instead of chromatographing the final product, it was fractionated in absolute methanol at temperatures between 0° and −78°C. following a method described elsewhere (Milas, 1939; Milas, Heggie and Mason, 1947). The product insoluble in methanol below −40°C. was recovered and examined spectroscopically. It was found to have a single band with a maximum at 321 mμ (Fig. 1, curve C) having a