VITAMINS AND HORMONES

ADVANCES IN RESEARCH AND APPLICATIONS

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Preface

The Editors are pleased to present this nineteenth volume of *Vitamins and Hormones*.

Six of the chapters in this volume are concerned with the hormones and only two with vitamins. The reverse occurred last year in Volume 18 when approximately seventy-five per cent of the book consisted of chapters on vitamins. Thus, the proportion of vitamin to hormone articles varies from year to year, but this does not reflect any trend in research activity. While there is still no evidence for a close similarity between the fundamental modes of action of the two groups of substances, their effects are closely interrelated at the metabolic level. In addition, the experimental approaches to them continue to have much in common.

Over the years, the Editors are continually impressed by the devotion of scientists who are willing to interrupt their research activities and their daily living so that they may serve their colleagues by preparing these critical reviews.

The Editors are always glad to receive suggestions of topics that may warrant review.

ROBERT S. HARRIS
DWIGHT J. INGLE

November, 1961

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Ubiquinones (Coenzymes Q), Ubichromenols, and Related Substances

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I. Introduction

The work to be described concerns lines of investigation which converged at the discovery of ubiquinones or coenzymes Q. The first was pursued in the Biochemistry Department of the University of Liverpool and the other in the Institute of Enzyme Research at the University of Wisconsin. Other laboratories were less directly concerned, and important contributions have since come from various quarters. It is now clear that a new chapter in the chemistry of natural products is taking shape and that advances are being made in understanding some fundamental biochemical processes.

The work at Liverpool developed from studies on the lipids found in intestinal mucosae and from observations of abnormally high concentrations of certain rat liver lipid constituents brought about by vitamin A deficiency. Two substances provisionally designated SA and SC were separated by chromatography of liver unsaponifiable matter on alumina. Each had a

distinctive ultraviolet absorption spectrum by the aid of which the separations could be followed. SA was found to be very widely distributed in animal tissues and in yeast; it was readily reduced and the product reoxidised. It was therefore given the name ubiquinone.

Later work indicated that although SC was less widely distributed than SA it was very closely related to it, and the name ubichromenol seemed appropriate.

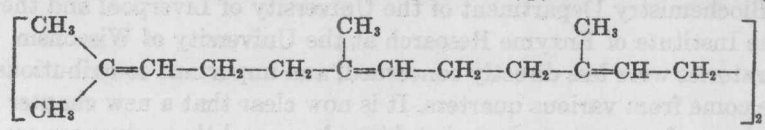
The work at Wisconsin grew out of studies on electron transport and in particular from investigating the lipid constituents of mitochondria obtained from heart muscle. The aim was to elucidate the roles of lipid cofactors in electron transport and oxidative phosphorylation. Today one thing at least is certain about this problem, namely that it is both complicated and difficult. The Wisconsin workers isolated a new substance known at different stages as Q_{275}, mitoquinone, and coenzyme Q.

The two groups of investigators were concerned with the same substance, or as it turned out, the same group of substances. As a new pattern of knowledge emerged, it became evident that recent studies on the tocopherols, the vitamins K, and other substances could not be left out of the reckoning. Instead of two lines of research meeting, several lines were in fact converging. This does not, however, mean that the pieces will easily fall into place; much remains to be done and only an interim report is possible.

II. The Chemical Nature of some Relevant Minor Constituents of Lipids

1. Hydrocarbons

a. Squalene. Squalene ($C_{30}H_{50}$) is widely distributed (in small amounts) in animal tissues and is of theoretical interest as the precursor of cholesterol on a biosynthetic route which includes: mevalonate, squalene, lanosterol, and cholesterol. Its structure (I) (which can be abbreviated ip ip ip pi pi pi) shows an irregular arrangement of six isoprenoid (ip) residues so as to per-



(I)

mit a symmetrical molecule to be formed. The biosynthetic step by means of which there is a change of linkage in the middle of the molecule is obviously of great importance since it determines the appearance not only of squalene, but of cholesterol and substances derived from it. The cyclization of squalene to give lanosterol is an oxidative process as also is the synthesis of cholesterol from lanosterol. It is perhaps worth noting that the fish liver

oils richest in squalene (i.e. those from certain elasmobranch fishes) are practically devoid of vitamin A. This absence of vitamin A may be a cause of the accumulation of squalene. Squalene is the most important hydrocarbon constituent of human sebum (Boughton *et al.*, 1955). As much as 10% of adult sebum is squalene and there is about half as much in the sebum from children. Straight-chain paraffins formerly thought to be present in human sebum are now regarded as more likely to be contaminants.

b. Hepene. Channon and Marrian (1926) and Channon *et al.* (1934) isolated from the unsaponifiable matter of pig liver an unsaturated compound believed to have the formula $C_{45}H_{76}$ or $C_{50}H_{84}$ (29 mg./100 gm. liver). Dimter (1941, 1942) confirmed the work of Channon *et al.*, and his findings favored the formula $C_{45}H_{76}$ with 8 double bonds instead of 9. Dimter gave the name hepene to this compound. Little attention has been given to hepene for about twenty years, and in fact it is not easy to obtain it. "Hepene" may have been an artifact derived from dolichol (Section 2c) or a related substance such as solanesol or an ubiquinone (Hemming *et al.*, 1960).

The carotenoid hydrocarbons will not be discussed as they do not at present seem to have a direct bearing on the topic under review.

2. Alcohols

a. Phytol. Phytol ($C_{20}H_{39}OH$) is a familiar compound as a constituent of chlorophyll. It has a regular structure whereas the more unsaturated "xanthophylls" have the irregular structure in that the mode of linkages of the isoprene residues is reversed at carbon atoms 15 and 15′. It is this which determines symmetry in carotenoids.

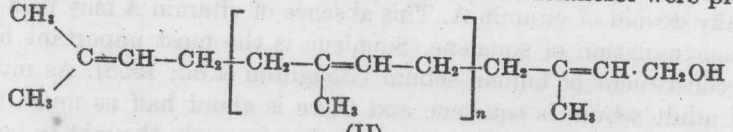
b. Solanesol. An unsaturated alcohol of low melting point, solanesol was discovered in tobacco (Rowland *et al.*, 1956). The processing to tobacco leaves does not result in appreciable destruction of solanesol, and the amount present is about 0.4% of the dry weight. The infrared absorption spectrum showed a close qualitative resemblance to that of farnesol (Plina and Sorm, 1950). Catalytic hydrogenation gave rise to a saturated alcohol and, in some experiments, to a saturated hydrocarbon. Solanesol showed a C—OH vibration in the infrared at 10μ, and in the saturated alcohol there was a displacement to 9.5μ. A similar shift occurred when farnesol, geraniol, and phytol were reduced and was ascribed to the change:

$$-CH_2-\underset{\displaystyle CH_3}{\overset{\displaystyle |}{C}}=CH-CH_2OH \rightarrow -CH_2-\underset{\displaystyle CH_3}{\overset{\displaystyle |}{CH}}-CH_2CH_2OH$$

The saturated alcohol was readily oxidized to the corresponding acid.

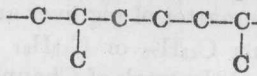
Solanesol was found to contain only one isopropylidene group; from the foregoing evidence, supported by molecular weight determinations, elemental analyses, and quantitative measurements of unsaturation, formula

(II) was reached ($n = 8$). Four different esters of solanesol were prepared

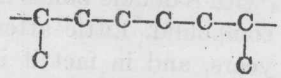


(II)

and their properties agreed best with $n = 8$. The isoprene chain was unsymmetrical, i.e. followed the regular arrangement



and not the irregular arrangement at the center of the squalene molecule.



More recently Erickson *et al.* (1959) and Kofler *et al.* (1959c) have proved that the formula of solanesol is $C_{45}H_{73}OH$ and not $C_{50}H_{81}OH$. The molecular weight (Kofler *et al.*, 1959c) was fixed by making solanesyl acetate-*1*-C^{14} and the purified product was recrystallized until the radioactivity became constant. The same sample of C^{14}-labeled acetic anhydride was used to prepare β-naphthylacetate-*1*-C^{14}. With this material, similarly recrystallized, as a reference standard, the isotope dilution method indicated for solanesyl acetate a molecular weight of 674 ± 6 (theory for $C_{45}H_{73}O \cdot COCH_3 = 673$). Various other esters were prepared by both groups of workers. Solanesol was found to yield the corresponding aldehyde by leaving it to stand in petrol over solid manganese dioxide (cf. Ball *et al.*, 1947b), and spectrophotometric determinations on the 2,4-dinitrophenylhydrazones of farnesaldehyde and solanesaldehyde helped to fix the molecular weight. As will be seen later this is a matter of some significance. The formula $C_{45}H_{73}OH$ and the structure have now been confirmed by total synthesis (Ruëgg *et al.*, 1960).

There is as yet no reason to expect tobacco leaves to be the only—or even the best—plant source of solanesol, but the search for alternative sources has not been carried far.

Gloor and Wiss (1960) have found solanesol in human heart (4–6 μg./gm., i.e. 2–5 mg. per heart) and in human liver (20–50 μg./gm., i.e. 30–80 mg. per liver). The same authors (Gloor and Wiss, 1960) administered C^{14}-labeled mevalonic acid to rats and found that the liver solanesol, present only in very minor amounts, was not radioactive. They therefore suggest that the solanesol found in animal tissues comes from the food. This is an interesting conclusion because (as will be discussed later) the rat can synthesize the C_{45}-side chain of ubiquinone-45.

Solanesol can be determined by measuring the intensity of color produced on exposure to iodine vapor.

c. Dolichol ($C_{100}H_{161(?)}OH$; Pennock *et al.*, 1960). In the course of separating ubiquinone and ubichromenol from all the other constituents of the unsaponifiable matter of human kidney (Section X), a substantial fraction was collected which was eluted from alumina after ubiquinone but before ubichromenol. The infrared absorption spectrum suggested the presence of an isoprenoid alcohol. The material was therefore acetylated and again subjected to chromatography. The main acetate portion was crystallized and recrystallized from a mixture of ethanol and light petroleum. The product was then hydrolyzed by means of alkali, and the alcohol was isolated and crystallized.

The infrared spectrum of the alcohol resembled that of solanesol except

$$=\overset{|}{C}-CH_2OH$$

that the band at 10μ expected for an allylic grouping was lacking, whereas a C—OH vibration at 9.4μ was clearly displayed. Hydrogenation of both solanesol and the new alcohol gave perhydro derivatives exhibiting indistinguishable infrared absorption apart from differences in relative intensities of some bands. The differences suggested that dolichol was made up of molecules larger than those of solanesol. Both alcohols seemed to be primary.

Analyses and molecular weight determinations together with —C—CH_3 values pointed to a very long-chain isoprenoid monohydric alcohol and the name dolichol (Gr. *dolichos*, long) seemed suitable.

The *p*-phenyl azobenzoates of farnesol, cholesterol, solanesol, and dolichol were prepared and purified. For the first three the molecular weights were known and hence the molecular extinction coefficients for the ultraviolet absorption peak (due to the common *p*-phenyl azobenzoate chromophore) could be measured. They agreed very well and provided a means of determining the molecular weight of the dolichyl ester. Similar comparisons were made of the *p*-nitrobenzoates of phytol, farnesol, and dolichol. The degree of unsaturation of dolichol was determined from the iodine uptake, and the evidence *in toto* led to the conclusion that the alcohol contained 95 or 100 carbon atoms with 19 or 20 unconjugated double bonds.

Dolichol formed an aldehyde (with difficulty) when refluxed in a light petroleum solution with manganese dioxide for 2 hours (Ball *et al.*, 1947a, b). This aldehyde formed a 2,4-dinitrophenylhydrazone which was compared with the corresponding derivative of farnesaldehyde. This again supported the view that in dolichol the conjugated double bond (allylic) was lacking. Specially purified dolichol was converted (Isler *et al.*, 160) into the C^{14}-labeled acetate, and the molecular weight was accurately fixed at 1422 by isotope dilution. This has since been confirmed by mass spectrometry (unpublished work by Dr. R. I. Reed). The empirical formula

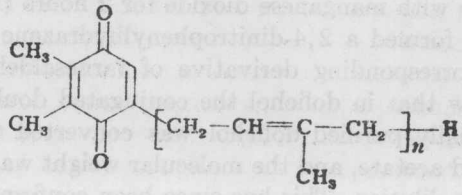
of dolichol is thus $C_{100}H_{161}OH$, but some details of structure have still to be decided.

Dolichol has been identified as a constituent of ox kidney, pig kidney and heart, rat liver, and other tissues. Its biological role has not been discovered, but its polyisoprenoid nature raises interesting queries.

3. Quinones

a. Kofler's Quinone. Kofler (1946) extracted dried lucerne (*Medicago sativa*) with petrol, and chromatographed the more soluble portion of the extract on acid-washed alumina, strongly deactivated by means of added water. Carotene was readily eluted by means of light petroleum, and chlorophyll was strongly held on the column. A fraction eluted after carotene by light petroleum was rechromatographed, and finally a crystalline product was obtained. It turned out to be a quinone quite distinct from vitamin K_1. It showed two ultraviolet absorption peaks at 254 and 263 mμ ($E^{1\%}_{1\,cm.}$ approximately 250) and there was also a much weaker maximum near 320 mμ. Reduction yielded a product with a single absorption band with its peak near 290 mμ. Comparison by Kofler of the absorption curve of his new quinone with those of a range of substituted quinones of known structure indicated that the structure was probably that of a trisubstituted *p*-benzoquinone. Molecular weight determinations, elemental analysis, and reductometric titration were all consistent with a large molecule of approximately 800 molecular weight. Lucerne contained about 150 mg. per kilogram dry weight, and oat plants, nettles, and ivy leaves were equally good as sources; pine needles were about twice as rich, and horse chestnut leaves gathered in autumn were the best source (0.6–1.0 gm./kg.) of the new quinone.

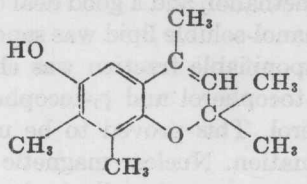
The substance was rediscovered by Crane and Lester (1958) and described by Crane (1959a,b). Two quinones were isolated from dried alfalfa (lucerne); one turned out to be ubiquinone (50) or coenzyme Q_{10} (Section V), and the other was identical with Kofler's quinone. The molecular weight of Q_{254}, as it was called, was about 770 and the absorption spectrum was consistent with the observed absence of methoxyl substituents. A polyisoprenoid side chain might account for the size of the molecule. Further investigation led to formula (III),



(III)

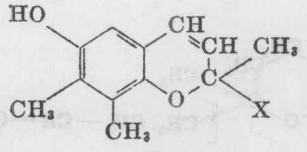
where $n = 10$ (Kofler *et al.*, 1959a,b), but in fact (Kofler *et al.*, 1959c) $n = 9$. One proof of this is that condensation of 2,3-dimethyl-1,4-hydroquinone with solanesol ($C_{45}H_{73}OH$) gave Kofler's quinone on oxidation. Erickson *et al.* (1959) also synthesized the quinone from solanesol and adduced other evidence concerning the structure. Nuclear magnetic resonance spectra did not at first prove satisfactory in distinguishing between $n = 9$ and $n = 10$, but this is within the compass of the method, given well-chosen reference compounds. Crane has given to Q_{254} the name plastoquinone because the substance is concentrated in the chloroplasts (see Section VI).

b. Solanachromene. This name was given by Rowland (1958) to a phenol which he isolated from aged, cured, tobacco leaf in which it was present to the extent of 0.5 gm. per kilogram dry weight. The compound is a colorless oil which solidifies at low temperature (m.p., 16–19°C.). The ultraviolet absorption spectrum resembled that of 2,2,4,7,8-pentamethyl-6-hydroxychromene (IV), λ_{max} mμ 233 (4.29), 264 (3.76), 272 (3.68), and 330 (3.65)



(IV)

(the values in parentheses are log ϵ, where ϵ is molecular extinction coefficient). Rowland's investigations suggested that solanachromene had structure (V).

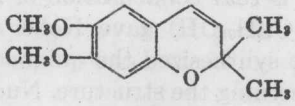


(V)

The elemental analyses pointed to X = $C_{46}H_{75}$, but it is more probable that solanachromene is related to Kofler's quinone and that X = $C_{41}H_{68}$. The infrared absorption spectrum closely resembled that of γ-tocopherol. Catalytic hydrogenation gave a product showing λ_{max} 296 mμ with a molecular extinction coefficient close to that of γ-tocopherol. Solanachromene is in fact isomeric with Kofler's quinone, and it remains to be seen whether the reductive cyclization occurs in the living plant or whether it results from the processing of tobacco leaf.

Alertsen (1955) isolated and characterized ageratochromene, a heterocyclic compound from the essential oils of some *Ageratum* species. Agerato-

chromene has structure (VI) and shows λ_{max} 280 mμ (ϵ, 5500) and 323



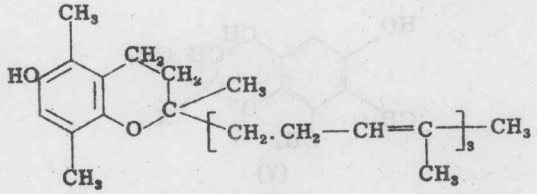
(VI)

(ϵ, 9300). It is readily reduced to the chroman with λ_{max} 293 mμ (ϵ, 6400). The existence of this compound in nature would fit the possibility that solanachromene may not be an artifact.

4. *Tocopherols*

J. Green *et al.* (1959, 1960c) determined the structure of ϵ-tocopherol from wheat bran. The raw material was extracted with light petroleum, and the resulting oil was chromatographed on alumina. Mixed tocopherols were eluted by 5% ethanol in light petroleum. The solvent was removed, the residue was dissolved in methanol, and a good deal of sterol was removed by crystallization. The methanol-soluble lipid was saponified in the presence of pyrogallol, and the unsaponifiable fraction was chromatographed on alumina. Benzene eluted α-tocopherol and ζ_1-tocopherol and 5% ethanol in benzene-eluted ϵ-tocopherol. This proved to be unsaturated and yielded β-tocopherol on hydrogenation. Nuclear magnetic resonance spectra indicated three olefinic hydrogens, a phenolic hydroxyl, two nonequivalent aromatic methyl groups, and finally a polyisoprenoid side chain made up to three isoprene units.

From this evidence, ϵ-tocopherol is believed to have structure (VII).



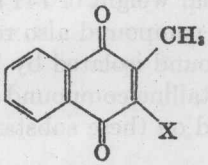
(VII)

ζ_1-Tocopherol is the analogous compound which gives α-tocopherol on reduction, and ζ_2-tocopherol is the 5,7-dimethyl derivative.

This work is of interest because it shows that although the classic tocopherols have a $C_{16}H_{33}$ saturated side chain related to phytol ($C_{20}H_{39}OH$), an unsaturated side chain, $-C_{16}H_{27}$, occurs in these compounds and may well be related to a natural alcohol, $C_{20}H_{31}OH$ (see also J. Green *et al.* 1960, a, b, c).

5. *Vitamin K_2*

The chemistry and biochemistry of the K vitamins (VIII) have been reviewed recently (Isler and Wiss, 1959). It is necessary here only to recapitulate briefly. Vitamin K_1 has for X a phytyl group, $—C_{20}H_{39}$; the classic



(VIII)

vitamin K_2 isolated by Doisy's team (McKee *et al.*, 1939; Binkley *et al.*, 1939, 1940) has been accepted as having for X a $—C_{30}H_{49}$ polyisoprenoid side chain. In fact, as Isler *et al.* (1958, a,b) showed, the original vitamin K_2 (m.p. 54°) had a $C_{35}H_{57}$ side chain, and a congener (m.p. 50°) had a $C_{30}H_{49}$ side chain. This interpretation was confirmed by unambiguous syntheses.

Francis *et al.* (1949) isolated a vitamin K_2-like substance from a strain of *Mycobacterium tuberculosis* as an uncrystallized oil. It showed the same type of ultraviolet absorption as the then known vitamin K_2, but $E^{1\%}_{1\,cm.}$ at 248 mμ was 241 (Snow, 1952). More recently Noll (1958) using *M. tuberculosis* (Brevannes) obtained a vitamin K_x (m.p. 58–59°) showing $E^{1\%}_{1\,cm.}$ 248 mμ = 240 and agreeing, in most of its properties with the compound of Francis *et al.* (1949). For a range of pure synthetic compounds of the vitamin K_2 type with polyisoprenoid side chains, ϵ_{max} at 248–249 mμ is about 18,900. If this applies to Noll's vitamin K_x, the molecular weight will be 18,900/24 = 787, the side chain X will account for 616, and $C_{45}H_{73}$ will correspond with 613. Noll at first favored a C_{40} side chain but later work (Noll *et al.*, 1960) confirmed the C_{45} side chain because the substance was synthesised from menadiol (2-methyl-1,4-naphthohydroquinone) and solanesol.

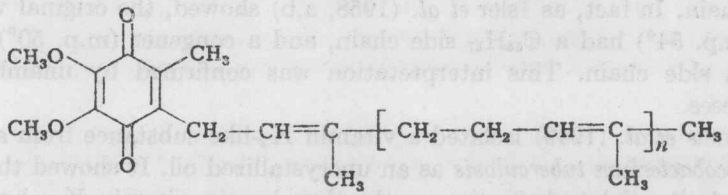
Martius and Esser (1958) studied the fate of 2-methyl-C^{14}-1,4-naphthoquinone fed to chickens and rats. They showed that it was converted to 2-methyl-3-(geranylgeranyl)-1,4-naphthoquinone. The steric configuration of this $C_{20}H_{33}$ side chain with its four unconjugated double bonds has not yet been decided. Martius regards the compound as the characteristic *animal* form of vitamin K just as vitamin K_1 is the plant form and the vitamins K_2 with side chains of 30, 35, 40, and 45 carbon atoms are characteristic of microorganisms.

Brodie *et al.* (1958) obtained evidence of another vitamin K in *Mycobacterium phlei*. The ultraviolet absorption spectrum, normal in shape, corresponded to the $E^{1\%}_{1\,cm.}$ values with a molecular weight of 620 but the infra-

red absorption spectrum did not entirely agree with either a K_1 or a K_2 type of side chain. There was, however, more of a resemblance to K_1 than K_2. Jacobsen and Dam (1960), on the other hand, obtained from *M. phlei* a vitamin K (m.p. −4°) showing $E^{1\%}_{1cm.}$ 248 mμ = 255, corresponding (18,900/25.5) with a molecular weight of 741 and a side chain of 570. From the infrared absorption this compound also resembled K_1 rather than K_2. The side chain of the compound isolated by Brodie *et al.* (1958) might be $C_{32}H_{65}$, and that of the crystalline compound of Jacobsen and Dam (1960), $C_{40}H_{81}$. More work is needed on these substances.

6. Ubiquinones and Ubichromenols

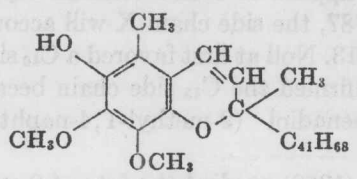
This introductory survey may be completed by anticipating (Section V) the general formula (IX) of the ubiquinones or coenzymes Q, *n* varying



(IX)

from 5 to 9 (C_{30}–C_{50}) depending on the natural product from which the individual substance was obtained.

Ubichromenol or SC (Section X) isolated from human tissue has structure (X) and is isomeric with ubiquinone-50. The ubiquinones and proba-



(X)

bly the ubichromenols resemble the vitamins K_2 in that polyisoprenoid side chains of varying length occur naturally.

III. Background of Studies on Ubiquinones

Lovern *et al.* (1937) found that intestinal mucosae of many species of fish were rich, sometimes very rich, in vitamin A. Thus the lipid of the tunica propria of halibut pyloric ceca contained as much as 40% of esterified vitamin A. It was found that retinene$_1$ (vitamin A aldehyde) could be reduced to vitamin A in the intestinal wall and that carotene could be converted to vitamin A in the intestinal epithelia (Ball *et al.*, 1947b; Glover