RECENT ISOPRENE CHEMISTRY[1]

PHYTOL, CAROTENOIDS, LIPOCHROMES, AND VITAMIN A

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INTRODUCTION

One would hardly have guessed that the delicious perfumes of acacia flowers or of orange blossoms had anything in common with the liver oils of the flounder, mackerel, or shark; that one of the products of the dry distillation of rubber was the chief building block used by nature in the construction of the growth-stimulating vitamin A, as well as of the green (chlorophylls) and yellow (xanthophylls) coloring matters of leaves, of the red pigments of annatto, the tomato, the Chinese Lantern plant, and the red pepper, of the yellow or orange pigments of carrots, dandelions, sunflowers, saffron, and yellow pansies, or of the brown pigments of some seaweeds; that the molecular configuration responsible for violet and orris perfumes likewise forms a part of the structure of the pigment of the carrot and of vitamin A; or that the coloring matter of egg yolks is a mixture of the yellow pigment found in corn with that which occurs in leaves. Yet this appears to be the case, and all these apparently unrelated substances seem to be built up from the same simple unit, isoprene (C_5H_8), a hydrocarbon heretofore regarded as peculiar to the vegetable kingdom but now shown to play an important rôle in the animal organism also.

In the following pages, it will be shown how the synthesis of farnesol and of nerolidol led first to that of phytol, then to that of squalene and of perhydrolycopene, to the determination of the

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probable constitution of carotene and of other carotenoids and lipochromes, and finally to a structural formula for vitamin A.

It may seem a far cry from the isoprene prepared by the destructive distillation of rubber or from calcium carbide, to the carotene which appears to be the immediate progenitor of the growth-producing vitamin A, or from the delicious perfumes of certain flowers to the squalene of shark liver oil which may be the actual parent of cholesterol; but the synthetic organic chemist has succeeded in showing how closely connected chemically are these apparently totally unrelated compounds. It certainly never entered the minds of Kerschbaum, Harries and Haarmann, Ruzicka, Verley, or any of the others who attacked the problem of the constitution of the flower alcohols, farnesol and nerolidol, that in discovering the answer to a question which seemed to be of interest only to the perfume industry, they would also find the key which would solve such other riddles as the structure of phytol, of squalene, of many other carotenoid and lipochrome pigments, and possibly of vitamin A. However, this is actually what has happened. To what further discoveries it may lead no one can yet say, for work along these lines is now going on actively in many laboratories,—organic, phytochemical, and biochemical.

PHYTOL

In an earlier paper Bogert (55) has given a resumé of the researches leading up to the establishment of the structural formulas of farnesol and nerolidol. It will be of interest first to explain how this knowledge was utilized to determine the constitution of phytol.

The investigations of Willstätter and others have shown that in the chloroplasts of plants there are four pigments or groups of pigments, in colloidal mixture with colorless substances of high molecular weight. These four groups of pigments are the chlorophylls (a and b)—the green pigments—and the carotenes and xanthophylls—the yellow ones.

Both chlorophylls contain at least two carboxyl groups, one of which is esterified with methyl alcohol and the other with phytol

When chlorophyll is treated with acids (e.g., alcoholic oxalic acid), it loses its magnesium and yields a neutral ester, phaeophytin, as a bluish-black wax of not very constant composition. Alkali hydrolysis of phaeophytin gives the unsaturated primary alcohol phytol (5, 6), $C_{20}H_{40}O$, whose iodide can be reduced by zinc dust to the olefin phytene, $C_{20}H_{40}$.

The experiments of Willstätter, Mayer and Hüni (11) showed that phytol could be reduced—electrolytically or catalytically—to phytanol (dihydrophytol) and to phytane, $C_{20}H_{42}$; the latter substance is obtained in better yield by the catalytic reduction of phytene. When a benzene solution of phytol is boiled with phthalic anhydride, phytadiene, $C_{20}H_{38}$, is formed. Oxidation of phytol by means of chromic oxide gave a mixture from which there was isolated a ketone whose formula was proved (16, 22) to be $C_{18}H_{36}O$. Oxidation of phytol with ozone gave this same $C_{18}H_{36}O$ ketone and glycolic aldehyde.

F. Gottwalt Fischer and K. Löwenberg (22, 32) finally correctly deduced the structure of phytol and corroborated it by the actual synthesis of this alcohol. Willstätter had suggested in the course of his work on phytol that it might be composed of four reduced isoprene units. With this hypothesis before them, and bearing in mind the configurations of geraniol and of farnesol, they concluded that the most likely structure would be:

$$Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_3CMe{:}CHCH_2OH$$

Oxidation of this compound should yield glycolic aldehyde and a $C_{18}H_{36}O$ ketone,

$$Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_3COCH_3$$
2,6,10-Trimethylpentadecan-14-one

which they succeeded in synthesizing as follows.

Farnesol, or its acetate, was reduced catalytically to hexahydrofarnesol.

$$Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_2OH$$
2,6,10-Trimethyldodecan-12-ol
Hexahydrofarnesol

The bromide of hexahydrofarnesol was then condensed with sodium acetoacetate. Saponification of the product

$$Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_2CH(COOR)COCH_3$$
2,6,10-Trimethylpentadecan-14-one-13-acid

gave a ketone identical with the one prepared by the oxidation of phytol.

This synthesis was corroborated by a second one, the successive steps in which were as follows:

By catalytic reduction, pseudoionone was changed into its hexahydro derivative, and the latter was treated with acetylene and sodamide at a low temperature. The product (dimethylethinylundecanol) was converted by the addition of two hydrogen atoms into tetrahydronerolidol. When the latter was heated for ninety hours with acetic anhydride at 97–98° and the product was saponified, it was isomerized to tetrahydrofarnesol. The bromide of tetrahydrofarnesol was then condensed with sodium acetoacetate and the product was hydrolyzed to the trimethylpentadecenone, reduction of which by hydrogen in the presence of palladized calcium carbonate gave the same $C_{18}H_{36}O$ ketone as was obtained by the other method of synthesis.

Synthesis of the ketone, $C_{18}H_{36}O$, *from pseudoionone*

$$Me_2C{:}CH(CH_2)_2CMe{:}CHCH{:}CHCOCH_3 + H_2 \rightarrow$$
Pseudoionone

$$Me_2CH(CH_2)_3CHMe(CH_2)_3COCH_3 + C_2H_2 + NaNH_2 \rightarrow$$
Hexahydropseudoionone or
2, 6-dimethylundecan-10-one

$$Me_2CH(CH_2)_3CHMe(CH_2)_3CMe(OH)C{:}CH + H_2 \rightarrow$$
2, 6-Dimethyl-10-ethinylundecan-10-ol

$$Me_2CH(CH_2)_3CHMe(CH_2)_3CMe(OH)CH{:}CH_2 + Ac_2O \rightarrow$$
Tetrahydronerolidol or
2, 6-Dimethyl-10-vinylundecan-10-ol

$$Me_2CH(CH_2)_3CHMe(CH_2)_3CMe{:}CHCH_2OH + PBr_3 \rightarrow$$
Tetrahydrofarnesol

$Me_2CH(CH_2)_3CHMe(CH_2)_3CMe{:}CHCH_2Br + NaCHAcCOOR \rightarrow$
Bromide of tetrahydrofarnesol

$Me_2CH(CH_2)_3CHMe(CH_2)_3CMe{:}CHCH_2CH(COOR)COCH_3 \xrightarrow{\text{hydrolysis}}$

$Me_2CH(CH_2)_3CHMe(CH_2)_3CMe{:}CH(CH_2)_2COCH_3 + H_2 \rightarrow$
2, 6, 10-Trimethyl-10-pentadecen-14-one

$Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_3COCH_3$
2, 6, 10-Trimethylpentadecan-14-one

With the constitution of the $C_{18}H_{36}O$ ketone established, Fischer and Löwenberg proceeded to the synthesis of phytol itself, using this ketone as initial material. The ketone was condensed with acetylene, the acetylene bond was then reduced catalytically to an olefin bond, and the product was isomerized, by heating it with acetic anhydride, to the tetramethylhexadecenol which was identical with natural phytol.

Synthesis of phytol

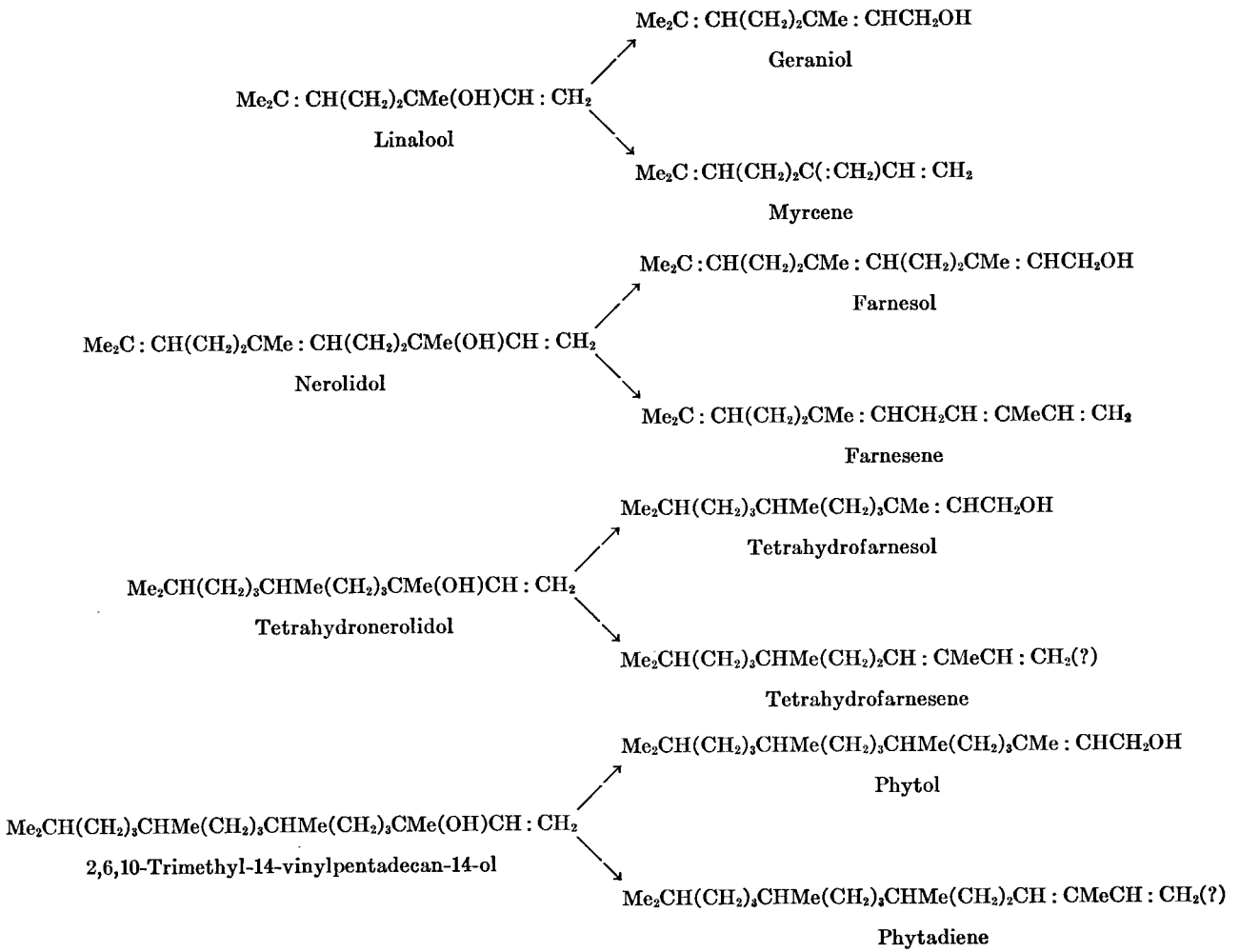
$Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_3COCH_3 + C_2H_2 + NaNH_2 \rightarrow$
2, 6, 10-Trimethylpentadecan-14-one

$Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_3CMe(OH)C{:}CH \xrightarrow[\text{reduction}]{\text{catalytic}}$
2, 6, 10-Trimethyl-14-ethinylpentadecan-14-ol

$Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_3CMe(OH)CH{:}CH_2 + Ac_2O \rightarrow$
2, 6, 10-Trimethyl-14-vinylpentadecan-14-ol

$Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_3CMe{:}CHCH_2OH$
Phytol

When digested with acetic anhydride, linalool and other compounds of the same type undergo both rearrangement and dehydration. Thus, linalool yields not only geraniol (i.e., geranyl acetate) but also myrcene; nerolidol yields farnesol and farnesene; tetrahydronerolidol yields tetrahydrofarnesol and tetrahydrofarnesene; and 2,6,10-trimethyl-14-vinylpentadecan-14-ol gives phytol and phytadiene, the latter substance being identical with the $C_{20}H_{38}$ obtained from natural phytol by the action of phthalic anhydride in benzene solution.


Me2C : CH(CH2)2CMe : CHCH2OH
Geraniol
Me2C : CH(CH2)2CMe(OH)CH : CH2
Linalool
Me2C : CH(CH2)2C(:CH2)CH : CH2
Myrcene
Me2C : CH(CH2)2CMe : CH(CH2)2CMe : CHCH2OH
Farnesol
Me2C : CH(CH2)2CMe : CH(CH2)2CMe(OH)CH : CH2
Nerolidol
Me2C : CH(CH2)2CMe : CHCH2CH : CMeCH : CH2
Farnesene
Me2CH(CH2)3CHMe(CH2)3CMe : CHCH2OH
Tetrahydrofarnesol
Me2CH(CH2)3CHMe(CH2)3CMe(OH)CH : CH2
Tetrahydronerolidol
Me2CH(CH2)3CHMe(CH2)2CH : CMeCH : CH2(?)
Tetrahydrofarnesene
Me2CH(CH2)3CHMe(CH2)3CHMe(CH2)3CMe : CHCH2OH
Phytol
Me2CH(CH2)3CHMe(CH2)3CHMe(CH2)3CMe(OH)CH : CH2
2,6,10-Trimethyl-14-vinylpentadecan-14-ol
Me2CH(CH2)3CHMe(CH2)3CHMe(CH2)2CH : CMeCH : CH2(?)
Phytadiene

Phytol contains both an olefin bond and two asymmetric carbon atoms. Hence it may exist in *cis-trans* and in optical isomers.

CAROTENOIDS AND LIPOCHROMES[2]

Wackenroder (1), just one hundred years ago, isolated from the carrot the first pigment of this class and named it "carotin." Later, Tswett (10) suggested the name "carotenoid" for the whole class of related pigments; this name has been generally adopted. Since we know that the name "carotin" would signify a hydrocarbon, "carotene" is now used instead. This is in harmony with our present system of nomenclature.

The carotenoids occur also in the *corpus luteum* of cattle, in the serum and body fat of many animals, in the yolk of hens' eggs, in the skin of man and beast, and elsewhere in the animal organism. The function of these pigments in plants is still obscure. Animals apparently get these coloring matters entirely from their plant food and are unable to synthesize them themselves. Because of their solubility in fats, they tend to accumulate therein, and, as they become concentrated, they impart their color to the fats. Hence Salkowski (3) favors the familiar term "lipochromes." These substances are gradually eliminated from the animal organism unchanged.

It was not until three or four years ago that the assault upon the problem of the constitution of these pigments began to make real headway. Since then it has advanced with increasing speed, as knowledge of the field has expanded, methods of obtaining the natural products in sufficient amount and purity have been improved, and new recruits have joined the ranks of the investigators.

It seems clear now that most, if not all, of the carotenoids are composed of isoprene units. They differ from the terpenes and camphors in that while the latter are formed by simple polymerization or condensation of isoprene units, the genesis of

[2] For an excellent review of this subject, covering the period prior to 1922, the reader is referred to "Carotenoids and Related Pigments," by Leroy S. Palmer, American Chemical Society Monograph Series, Chemical Catalog Co., Inc., New York (1922).

the carotenoids from such units is accompanied by considerable dehydrogenation, resulting in the formation of numerous double bonds, often in conjugated systems.

In the terpenes, sesquiterpenes, and diterpenes, there apparently exists a chain of normally conjugated isoprene units, resulting from the successive linear, "head to tail" union of two, three, or four isoprene molecules. In the case of the triterpene, squalene, and of the carotenoids derived from tetraterpenes, however, this building plan has not been continued; these larger molecules seem to be formed by the union of two of the shorter chains (e.g.,

TABLE 1

Simple carotenoids and lipochromes

NAME	MOLECULAR FORMULA	DOUBLE BONDS	ACYCLIC OR CYCLIC
Squalene	$C_{30}H_{50}$	6	Acyclic
Lycopene	$C_{40}H_{56}$	13	Acyclic
Carotenes	$C_{40}H_{56}$	11	Cyclic
Vitamin A	$C_{20}H_{30}O$	5	Cyclic
Leaf xanthophylls	$C_{40}H_{56}O_2$	11	Cyclic
Lutein (?)	$C_{40}H_{56}O_2$	11	Cyclic
Zeaxanthin	$C_{40}H_{56}O_2$	11	Cyclic
Capsanthin	$C_{35}H_{50}O_3$ (?)		Cyclic
Taraxanthin	$C_{40}H_{56}O_4$		Cyclic
Violaxanthin	$C_{40}H_{56}O_4$		Cyclic
Fucoxanthin	$C_{40}H_{56}O_6$	10	Cyclic
Crocetin (alpha-)	$C_{20}H_{24}O_4$	7	Acyclic
Bixin	$C_{25}H_{30}O_4$	9	Acyclic
Azafrin	$C_{28}H_{40}O_4$ (?)	7	Acyclic

farnesol, phytol, etc.), with production of a chain which cannot increase in length by similar processes. It is doubtful, therefore, that isoprene derivatives of higher molecular weight, such as rubber, whose molecule Staudinger believes may contain 1000 isoprene residues, are made up of chains of isoprene units in normal conjugated systems. In fact it is not at all unlikely that they are built up on a totally different plan.

In table 1 are listed the simple carotenoids and lipochromes which have been described to date. These may occur in nature, either free or in combination (generally as esters or glucosides).

On scanning this list, the interesting fact will appear that the formula for each one of these compounds, with the exception of azafrin (whose formula is still very much in doubt), is some multiple of C_5.

Squalene or spinacene, $C_{30}H_{50}$

In the liver oil of sharks, and of certain other elasmobranch fish, there occurs a compound known as squalene, which has been proven to be identical with the "spinacene" discovered by Chapman (15) in the liver oils of certain deep-sea fish caught off the Moroccan coast.

The work of Heilbron and his associates (30, 31) has shown that this substance is a dihydrotriterpene, of the formula $C_{30}H_{50}$. It adds six molecules of hydrogen and hence must be an acyclic hydrocarbon with six olefin bonds.

On the basis of the products formed by the oxidation of squalene and of some of its hydro derivatives, as well as upon other reactions, Heilbron proposed several unsymmetrical formulas, no one of which was altogether satisfactory. One of the products which he obtained by ozonolysis of partly reduced squalene was a ketone, $C_{19}H_{38}O$. By synthesizing this ketone from hexahydrofarnesol, Karrer (43) proved it to be 2,6,10-trimethylhexadecan-15-one.

$Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_2OH \rightarrow$
Hexahydrofarnesol

$Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_2Br + Mg \rightarrow$

$Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_2MgBr + ClCH_2OMe \rightarrow$

$Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_2CH_2OMe + HBr \rightarrow$

$Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_2CH_2Br + NaCH(COOR)Ac \rightarrow$

$Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_2CH_2CH(COOR)COCH_3 \rightarrow$

$Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_2CH_2CH_2COCH_3$
2,6,10-Trimethylhexadecan-15-one

Karrer (43) then suggested a symmetrical formula for squalene. He and Helfenstein (56) demonstrated the correctness of this deduction by the synthesis of squalene from farnesol, pointing

out at the same time how such a chain might originate by benzoin condensation or by the pinacone reduction of two moles of farnesal.

Synthesis of squalene

$$Me_2C{:}CH(CH_2)_2CMe{:}CH(CH_2)_2CMe{:}CHCH_2OH + PBr_3 \xrightarrow{5^\circ}$$
Farnesol

$$Me_2C{:}CH(CH_2)_2CMe{:}CH(CH_2)_2CMe{:}CHCH_2Br + Mg \text{ (or K)} \rightarrow$$
Farnesyl bromide

$$Me_2C{:}CH(CH_2)_2CMe{:}CH(CH_2)_2CMe{:}CHCH_2$$

$$Me_2C{:}CH(CH_2)_2CMe{:}CH(CH_2)_2CMe{:}CHCH_2$$
Squalene

$$2\ Me_2C{:}CH(CH_2)_2CMe{:}CH(CH_2)_2CMe{:}CHCHO \rightarrow$$
Farnesal

$$Me_2C{:}CH(CH_2)_2CMe : CH(CH_2)_2CMe : \begin{matrix}CH{-}CH(OH)\\ |\\ CH{-}CO\end{matrix} \quad \text{or} \quad \begin{matrix}{-}CH(OH)\\ |\\ {-}CH(OH)\end{matrix} \rightarrow$$

$$Me_2C{:}CH(CH_2)_2CMe{:}CH(CH_2)_2CMe{:}\begin{matrix}CHCH_2\\ |\\ CHCH_2\end{matrix}$$
Squalene

In this synthesis, the yields were low because of the impossibility of securing a pure farnesyl bromide. Both farnesyl and nerolidyl bromides tend to change partly into one another by the reversible reaction

$$-CMe{:}CH\cdot CH_2Br \rightleftarrows -CMeBr\cdot CH{:}CH_2$$

so that squalene can be prepared by starting with either farnesol or nerolidol. The synthesis of squalene is the first synthesis of a naturally occurring triterpene.

Heilbron, Kamm, and Owens (17) call attention to a possible relationship between this hydrocarbon, stigmasterol ($C_{30}H_{50}O$), and cholesterol ($C_{27}H_{46}O$), and report that feeding experiments carried out on rats by H. J. Channon, of University College, London, showed that the administration of squalene resulted in the cholesterol content of the liver being more than doubled.

This indicates that squalene may be a precursor in cholesterol synthesis in the animal body, perhaps, as Karrer (56) has suggested, by multiple cyclization and demethylation of the squalene molecule. Further, Andre and Canal (29) have found that in young and old sharks the cholesterol and squalene appear to bear a reciprocal relation, inasmuch as in young fish the cholesterol predominates, whereas in old ones there is more squalene than cholesterol.

Lycopene (lycopin), $C_{40}H_{56}$

The red coloring matter of the tomato (*Lycopersicum esculentum*), known as lycopin (or, better, lycopene), was first isolated in the pure condition by Willstätter and Escher (8); its molecular formula was shown to be $C_{40}H_{56}$.

Under proper conditions, lycopene will add thirteen moles of hydrogen and the perhydrolycopene, $C_{40}H_{82}$, so obtained shows by its molecular refraction and its other properties that it belongs to the paraffin series. Lycopene itself therefore must be an acyclic hydrocarbon carrying thirteen double bonds (or their equivalent). Hydrolysis of lycopene ozonide gave large amounts of acetaldehyde and of acetic acid, as well as acetone. The presence of the latter is evidence for a terminal Me_2C: group.

If we assume that lycopene is built up of eight isoprene rests, there would be six intermediate $-C_5H_6-$ groups, and two terminal $-C_3H_7$ groups, making a total of $C_{40}H_{50}$, and leaving six hydrogen atoms to be accounted for, presumably by formation of saturated linkages. If these saturated carbon atoms were grouped at one end of the chain, ozone cleavage should give higher fatty acids than those isolated. Hence all thirteen double bonds cannot be conjugated. However, the deep color of lycopene makes it likely that its molecule contains a still larger number of conjugated double bonds than either crocetin or bixin, where it has been shown that deep color requires more than four conjugated double bonds. On the basis of these considerations, an unsymmetrical formula was proposed for lycopene, in which the location of the CH_2 groups was unsettled; however, it was regarded as unlikely that any carbon atom carrying a methyl side chain was

hydrogenated, for it would then constitute a center of asymmetry leading to optical activity, while hydrogenated lycopene is optically inactive.

Further study of the problem, however, convinced Karrer (43, 57, 64) that the lycopene formula should be symmetrical rather than unsymmetrical. He justified this conclusion by the synthesis, from dihydrophytyl bromide and potassium, of a perhydrolycopene, $C_{40}H_{82}$, a 2, 6, 10, 14, 19, 23, 27, 31-octamethyl-*n*-dotriacontane, which agreed in its properties with the perhydrolycopene obtained by the hydrogenation of lycopene itself.

Synthesis of perhydrolycopene

$$\underset{\text{Dihydrophytol}}{Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_2OH} \rightarrow$$

$$\underset{\text{Dihydrophytyl bromide}}{Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)Br} + K \rightarrow$$

$$(Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_2)_2 = \underset{\text{Perhydrolycopene}}{C_{40}H_{82}}$$

Such a structure for lycopene explains satisfactorily its optical inactivity, the fact that when oxidized with potassium permanganate it yields only succinic acid and about 4.5 moles of acetic acid, that when treated with chromic oxide it yields six moles of acetic acid, and that upon ozonolysis 80 per cent of the calculated amount of acetone is obtained.

The explanation of the apparent interruption, in the middle of the chain, of the usual conjugation of the isoprene units, is that this sequence of four CH groups is due to the probable formation of such a chain from two moles of a C_{20} chain as indicated below.

Possible origin of lycopene

$$2\ \underset{\text{Phytol aldehyde}}{Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_3CMe:CHCHO} \xrightarrow[\text{or pinacone reduction}]{\text{by benzoin condensation}}$$

$$\begin{array}{l} Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_3CMe:CH{-}CH(OH) \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad | \\ Me_2CH(CH_2)_3CHMe(CH_2)_3CHMe(CH_2)_3CMe:CH{-}CO \end{array} \quad \text{or} \quad \begin{array}{l} {-}CH(OH) \\ \quad | \\ {-}CH(OH) \end{array} \rightarrow$$

$$\underset{\text{Lycopene}}{(Me_2C:CH(CH_2)_2CMe:CHCH:CHCMe:CHCH:CHCMe:CHCH:)_2}$$

Carotene, $C_{40}H_{56}$

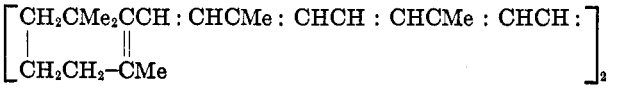
Carotene itself is quite widely distributed in both vegetable and animal kingdoms. For reasons which will appear as we proceed, it is perhaps the most interesting member of the group.

Its molecular formula was established by Willstätter (7) as $C_{40}H_{56}$.

In 1928, Zechmeister, Cholnoky and Vrabely (27), by catalytic reduction, succeeded in adding eleven moles of hydrogen to carotene, and thereby obtained a $C_{40}H_{78}$ compound, which behaved as though saturated, and hence probably contained two cycles in its molecule. From this they reasoned that carotene itself also probably contained two such cycles.

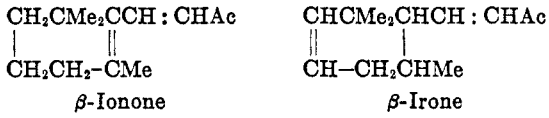
Karrer has shown that the ozonolysis of carotene does not yield any acetone; this indicates the absence of terminal $:CMe_2$ groups. Furthermore, the hydrocarbon is optically inactive. Karrer and his coworkers (35, 37, 38, 40, 43) also found that when carotene was oxidized by potassium permanganate, β-ionone was formed first and then the usual oxidation products of this ionone, namely, dimethylmalonic acid, α, α'-dimethylsuccinic acid, α, α'-dimethylglutaric acid, geronic acid, and 4.4 moles of acetic acid, but no isogeronic, butane-2, 2, 4- or pentane-2, 2, 5-tricarboxylic acids. When chromic oxide was the oxidizing agent, carotene gave six moles of acetic acid.

On the basis of these and other experimental observations, Karrer came to the conclusion that carotene, like the isomeric lycopene, possessed a symmetrical structure, in that both ends of the hydrocarbon chain were identical, and that carotene differed from its isomer in having the two ends of the lycopene chain cyclized. He therefore proposed (43) the following formula



β-Carotene
$C_{40}H_{56}$

and made the interesting suggestion that perhaps irone, the odorous principle of the orris root, and an isomer of ionone,



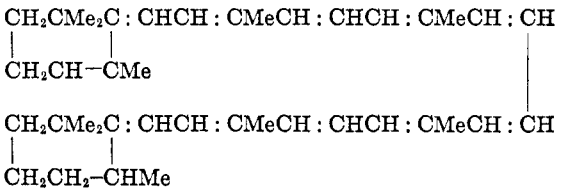
was formed in the plant by the oxidation of some carotenoid constituent. This Karrer formula has been supported also by ozonolysis experiments reported lately by Pummerer, Rebman, and Reindel (81).

In the United States, J. H. C. Smith (82) carried out some interesting experiments on the hydrogenation of carotene and found that one mole of hydrogen was added by aluminum amalgam in ether solution, and nine or ten moles of hydrogen by hydrogenation in the presence of the Adams catalyst. From these studies, he drew the conclusion that the gradual decrease in rotation on hydrogenation showed that the double bond hardest to saturate was that responsible for the asymmetry of the molecule and that the unsaturation reduced by the action of aluminum amalgam was probably attached to a ring. He proposed for carotene, therefore, a structure consisting of a straight chain of nine conjugated double bonds, which chain was conjugated further with two cyclopropane rings, thus resembling somewhat the formula suggested by Karrer, Helfenstein, Wehrli, and Wettstein (43).

Kuhn and Lederer (75, 79) have very recently succeeded in separating carotene into an α- and a β-carotene. From carotene tetraiodide and mercury (or sodium thiosulfate), they prepared an isocarotene, apparently closely related to β-carotene but not as yet discovered in nature. All three carotenes possessed the $C_{40}H_{56}$ formula.

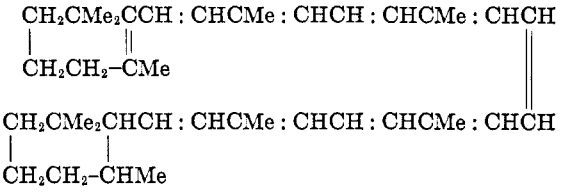
Karrer, Helfenstein, Wehrli, Pieper, and Morf (58) have also succeeded in separating natural carotene into a dextrorotatory α-form (m.p. 170°) and an optically inactive β-isomer (m.p. 181–2°). The structure given previously (p. 277) is ascribed to the β-form. It is possible that α-carotene is itself non-homogeneous, for the separation of such mixtures is exceedingly difficult, and until this question is decided its correct constitutional formula must remain in doubt.

However, if this α-carotene is really a chemical individual, Karrer and Morf (60) believe that it carries an olefin bond between carbon atoms 3 and 4 in one ring, with an asymmetric carbon atom in the other ring, the structure of this latter cycle being uncertain. They propose, as the most plausible formula for α-carotene, the following structure.



α-Carotene
$C_{40}H_{56}$

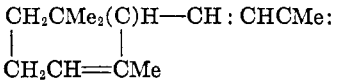
For the bright yellow dihydro derivative (82) formed by reduction with aluminum amalgam in moist ether solution they propose the structure



Dihydro-α-carotene
$C_{40}H_{58}$

since aluminum amalgam attacks conjugated but not unconjugated olefin bonds, and would act on the ends of this multiple ethylenic system with a consequent general rearrangement of the unsaturation.

Kuhn and Lederer (75) point out that the presence of the structure



would account for the asymmetric (C) believed to exist in the α-carotene molecule.

With potassium permanganate, α-carotene yields α,α'-dimethylsuccinic acid, while its dihydro derivative gives α,α'-dimethylglutaric acid. Hence the ring double bond in the latter is at 2, 3; this explains the formation of geronic acid from the dihydro derivative on ozonolysis. It also explains why ozonolysis of crude carotene gives dimethylglutaric, but no isogeronic acid.

Studies of the absorption spectra (72) of solutions of α- and β-carotenes confirmed the assumption of their non-identity, indicated that the β- form was symmetrical, that the α-isomer contained an asymmetric carbon atom in one or both rings, and that the chain of conjugated double bonds was the same in both.

A dihydro derivative was prepared also by the action of aluminum amalgam upon a moist ether solution of β-carotene (62).

Pure, optically inactive, β-carotene (65), like the natural carotene mixture, yields geronic acid on ozonolysis. Comparison of the yields of this acid from β-ionone and from this β-carotene, under identical conditions, support the assumption of the presence of two β-ionone rings in β-carotene and thus corroborate the symmetrical formula already devised.

When this pure β-carotene was reduced by aluminum amalgam to the dihydro derivative and the latter was ozonized, 18.5 per cent of the calculated (assuming two β-ionone rings to be present) amount of geronic acid was obtained, showing that the hydrogen had not been added, as assumed by Kuhn and Winterstein (25), at the ends of the conjugated system of olefin bonds, possibly because of the stereointerference of the side chains.

Since certain color reactions which have been used to show the presence of vitamin A are given also by carotenoids, experiments were conducted to ascertain whether or not these latter had any growth-stimulating properties when administered to animals. The only ones found to exhibit this property were carotene and xanthophyll. In fact, the vitamin A action of highly purified carotene is very great. The recent experiments in this field by von Euler, Demole, Karrer, and Walker (42) led to the conclusion that the active growth-stimulating principle of plants was either carotene itself or a compound which was chiefly or wholly com-

bined with carotene and not separated from it by crystallization. However, in the case of cholesterol, as is known, 100 recrystallizations do not remove the last portions of ergosterol, the mother-substance of the antirachitic vitamin. Since vitamin A activity runs parallel with carotene content in plants, determination of the latter can be used to estimate the former. Vitamin A of man and animals apparently is not identical with carotene, but may owe its genesis to the influence of carotene or to the rearrangement of the latter.

When fed to rats, at the rate of 0.01 mg. per day, the daily growth was <0.75 gram for the α- and 1.0 gram for the β-isomer (72). The dihydro-α- and β-carotenes also exhibited strong growth-stimulating properties (60, 62).

In a recent article, Ahmad (51) states: "There is now ample evidence that administration of carotene to animals fed on vitamin A-deficient diet is followed by a return to normal health and the appearance in the liver of a substance showing the properties of vitamin A." In feeding experiments on rats, he noted the formation from carotene of a substance resembling vitamin A, but was unable to show this in the case of cats, or in experiments carried out *in vitro* with either intestinal bacteria or liver tissue.

Olcott and McCann (80), however, were more successful in that they found that carotene was changed to vitamin A by incubation *in vitro* with fresh liver tissue, or with an aqueous extract of liver, by what appeared to be an enzyme, for which they proposed the name "carotenase."

Since vitamin A deficiency is often accompanied by anemia, Binet and Strumza (54) studied the hemoglobin-producing power of carotene. Administered to dogs, it caused an immediate and striking increase in the hemoglobin content of the blood.

Vitamin A from fish livers

Following up these discoveries in the carotenoid and lipochrome field, Karrer and his coworkers (66, 67, 71) are now engaged in a direct attack upon the constitution of vitamin A itself.

After laborious purification, a vitamin A preparation of 10,500 C. L. O. (cod-liver oil) units was obtained from the liver oil of

Hippoglossus hippoglossus (a Norwegian flounder). This preparation was ten times as active as carotene or Japanese biosterin in growth stimulation, and also in respect to the Carr-Price antimony trichloride reaction, which generally runs approximately parallel with growth stimulation.

By the action of aluminum amalgam upon its moist ether solution, it was reduced, although slowly and gradually, behaving in much the same way as carotene. On the other hand, it was much more resistant than most carotenoids to catalytic reduction, possibly owing to the presence of catalyst poisons.

The most interesting result was that obtained by ozonolysis, which yielded geronic acid, the amount of which increased with increase in the Lovibond figure of the material ozonized. From this it follows that the vitamin A of this liver oil contains the same carbon ring system as β-ionone and carotene,

$$\begin{array}{l} CH_2CMe_2CCH:..... \\ \quad\;|\qquad\quad\| \\ CH_2CH_2\text{–}CMe \end{array}$$

and in this sense is therefore a carotene derivative. The molecular weight of this vitamin A (of 10,500 C. L. O. units = over 100,000 blue values) was found to be 320 (in camphor). On the basis of this molecular weight, of the geronic acid yield on ozonolysis, and on the assumption of the existence of one β-ionone ring in the molecule, the content in pure vitamin A was calculated as 50 to 80 per cent. Apparently about 5 per cent of vitamin D was also present.

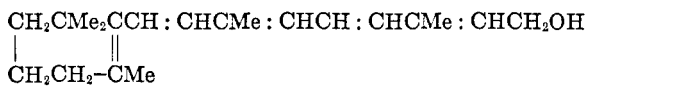
Oxidation by potassium permanganate gave considerable quantities of acetic acid, as in the case of the carotenoids. This speaks for the presence of :C·CMe: groups. The yield corresponded to 8.5 per cent of methyl carbon—as against 11.2 per cent in carotene—oxidized to acetic acid.

Since the color (an intense violet red) formed when this vitamin A preparation was treated with concentrated sulfuric acid resembled the color reaction obtained with dihydrocrocetin, Karrer thinks it probable that, like the latter, this vitamin A carries a conjugated system of six olefin bonds. Experiments have been undertaken, therefore, to synthesize compounds of this type.

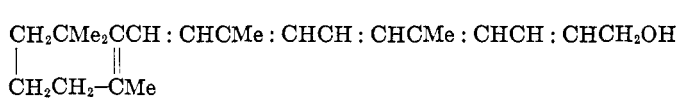
From other fish-liver oils, vitamin A preparations of still greater potency have been secured (67). From the liver oil of one of the mackerels (*Scombresox saurus*), a vitamin A fraction of high Lovibond value was separated as a thick, pale yellow, oil whose analysis, like that of the *Hippoglossus* vitamin A preparation, indicated a formula of $C_{20}H_{30}O$, or $C_{22}H_{32}O$, and which was apparently an alcohol, since esters could be prepared from it. Saponification of these esters by alcoholic alkali regenerated the original alcohol with all of its original properties. This is in agreement with the conclusions of Bacharach and Smith (21) that the vitamin A of cod-liver oil is an alcohol and that it exists in the liver as an ester.

Optical inactivity and feeding experiments demonstrated the absence in these vitamin A preparations of any appreciable amounts of the strongly optically active sterins or of vitamin D. Molecular weight values determined in camphor were between 300 and 320. Ozonolysis yielded larger amounts of geronic acid than the *Hippoglossus* preparation. Oxidation by potassium permanganate gave an amount of acetic acid corresponding to 9.7 per cent methyl carbon, and by chromic oxide an amount corresponding to 16.3 per cent. Catalytic hydrogenation added approximately five moles of hydrogen and gave a product whose analysis agreed with the formulas $C_{20}H_{40}O$ or $C_{22}H_{44}O$.

Based upon these experiments, the following constitutions are proposed for this vitamin A.



I ($C_{20}H_{30}O$)



II ($C_{22}H_{32}O$)

Scombresox vitamin A

Formula I, which represents an alcohol derived from half a carotene molecule, is the one preferred.

It is argued that the studies of these fish-liver oils demonstrate the presence therein of a polyene carrying the same carbon ring system and an acyclic side-chain similar to that of carotene, and that this polyene is either itself the cause of the blue coloration with antimony trichloride, or is at least one of the factors in this reaction. Since it has been shown, by Moore and by von Euler, that the liver of animals does not give this color reaction until after feeding the animals with carotene and that the substance isolated from livers possesses a carotenoid structure, it is not unreasonable to assume that it owes its formation there to a breakdown of the carotene molecule. Ahmad and Drummond (52) state that "colorimetric, spectroscopic, and biological examination showed that stores of vitamin A accumulate in the liver following administration of carotene."

Since, in general, Lovibond values and growth-stimulating power run parallel in such liver oils, it follows that this polyene has a growth-stimulating property, but that it alone is responsible for this effect of these liver oils remains to be proven.

Xanthophyll, $C_{40}H_{56}O_2$

In the chloroplasts of green leaves, in addition to carotene, another yellow pigment, xanthophyll, was separated in crystalline condition by Willstätter and Mieg (7), and its formula found to be $C_{40}H_{56}O_2$. On oxidation it yielded no acetone, and hence does not contain a terminal $:CMe_2$ group. Zechmeister (28) concluded that it was probably an acyclic compound carrying eleven olefin bonds.

Karrer and Jirgensons (45) succeeded in preparing its monomethyl, but not its dimethyl ether. Esters were easily prepared from it, by the action of acid chlorides and pyridine, and several (e.g., the dipropionate and the dipalmitate) have been described by Karrer and Ishikawa (44).

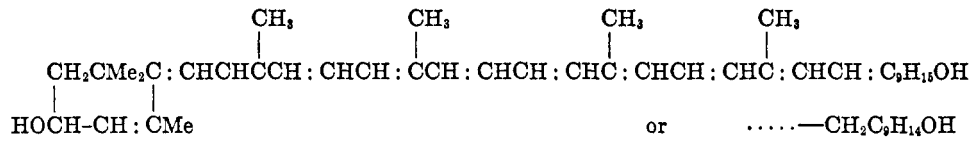
Kuhn, Winterstein, and Lederer (77) compared the xanthophylls from the leaves of a large number of plants and reported that one xanthophyll predominated (m.p. 193°; specific rotation in ethyl acetate, 145°), with only traces of others. They stated also that

they had separated the egg yolk lutein into this leaf xanthophyll and zeaxanthin, and proposed that the name "lutein" be retained for leaf xanthophyll rather than for the mixture present in egg yolk.

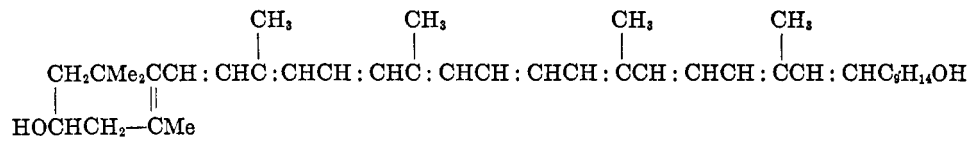
Kuhn (77) also suggested that the term "xanthophyll" be restricted to the C_{40} hydroxylated carotenoids, of which the following are now known: $(OH)_2$ = lutein (xanthophyll) and zeaxanthin; $(OH)_3$ = capsanthin; $(OH)_4$ = violaxanthin and taraxanthin; and $(OH)_6$ = fucoxanthin. These occur in nature either free or as esters.

Karrer, Helfenstein, Wehrli, Pieper, and Morf (58) point out that leaf xanthophyll, lutein (assuming that it is an individual and not a mixture), and zeaxanthin, are isomeric, all containing two alcoholic hydroxyl groups, but differing in optical activity. Like carotene, each possesses eleven olefin bonds, and yields four moles of acetic acid when oxidized by potassium permanganate and six moles when oxidized by chromic oxide. All three isomers with potassium permanganate give also dimethylmalonic acid and α, α'-dimethylsuccinic acid, but no α, α'-dimethylglutaric acid. The difference in the three isomers may be due to the different positions occupied by the hydroxyl groups or by the olefin bonds, to *cis-trans* isomerism, or to optical isomerism.

There should exist, therefore, as remarked by Karrer and Nilsson (63), xanthophylls corresponding to both α- and β-carotenes, whose optical activity would be due not only to their asymmetric $-CH(OH)-$ groups, but also, in the α-forms for example, to the presence therein of the same asymmetric carbon atom to which α-carotene itself owes its optical activity. In support of this hypothesis, they obtained by reduction of the xanthophyll of stinging nettles, a perhydroxanthophyll, $C_{40}H_{76}(OH)_2$, whose hydroxyl groups were replaced by bromine and the latter by hydrogen, with the formation of a saturated hydrocarbon, $C_{40}H_{78}$, which was optically active, whereas β-carotenel as noted above, is inactive. In direction and magnitude of rotation, it resembled its isomer, perhydrocarotene, and the original xanthophyll therefore is believed to be an α- and not a β-xanthophyll.



α-Xanthophyll



β-Xanthophyll

The hydroxyl group in the ring of known structure is probably in position 5, for if it were at 4 the compounds would be enols.

Von Euler, Karrer, and Rydbom (70) used a purified xanthophyll (m.p. 192°) obtained from the leaves of the stinging nettle and carefully freed from any possible carotene content. This was given to four rats in daily doses of 0.037 mg. It caused an increase in growth for four weeks; after this time the growth diminished and then ceased, but could be started up again by amounts of carotene insufficient alone to cause growth stimulation. These investigators hence conclude that carotene is not the only carotenoid concerned in the vital syntheses responsible for growth.

Lutein, $C_{40}H_{56}O_2$

This pigment of the yolks of hens' eggs, isomeric with the xanthophylls of leaves, differs from them in its low dextrorotation (+ 72°).

It has been discovered recently by Zechmeister and Tuzson (50) that it is the yellow pigment of sunflower sepals (*Helianthus annuus*), and that it exists there largely in the form of a crystalline ester. According to Kuhn and Lederer (78), it occurs also in the dandelion as a fatty acid ester.

Kuhn, Winterstein, and Lederer (77) have recently succeeded in separating the lutein of hens' egg yolks into leaf xanthophyll and zeaxanthin, as mentioned in the preceding section, but the complexity of the luteins of the sunflower and of the dandelion is still to be demonstrated.

Zeaxanthin, $C_{40}H_{56}O_2 = C_{40}H_{54}(OH)_2$

This crystalline yellow pigment from yellow corn (*Zea Mays*), although isomeric with xanthophyll and lutein, is quite different from them.

The researches of Kuhn and his associates (36, 47), and of Zechmeister and Cholnoky (48, 49), have made it clear that it contains eleven double bonds and two alcoholic hydroxyl groups, since it adds eleven moles of hydrogen when reduced catalytically in cyclohexane solution, and reacts with acid halides to form esters carrying two acyl groups per mole. Oxidized by chromic oxide, it gives six moles of acetic acid. With ferric chloride, the green color reaction of leaf xanthophyll is obtained.

In the berries of the sea-buckthorn (*Hippophaes rhamnoides*), there exists a pigment which Karrer and Wehrli (46) have shown to be an ester of zeaxanthin.

Physaliene, $C_{72}H_{116}O_4$

The physaliene, isolated by Kuhn and Wiegand (36) from the *Physalis*, alkekengi or Chinese Lantern plant, has been found by Zechmeister and Cholnoky (48) to be also the pigment of the red berries of the boxthorn (*Lycium halimifolium*).

Through the further researches of Kuhn, Winterstein, and Kaufmann (47), and of Zechmeister and Cholnoky (13), it has been assigned a molecular formula of $C_{72}H_{116}O_4$. It appears to be a zeaxanthin dipalmitate, or $C_{40}H_{54}(OCOC_{15}H_{31})_2$, since, when hydrolyzed by methyl alcoholic potassium hydroxide, it yields zeaxanthin and palmitic acid, and conversely, a compound similar to it has been synthesized from zeaxanthin, palmityl chloride, and pyridine. Other zeaxanthin esters were also synthesized (the dilaurate, distearate, etc.), and these investigators expressed the belief, which has since been justified, that many such "polyene waxes" would be discovered in nature.

Karrer and Pieper (61), however, observed that on ozonolysis physaliene yields some azelaic acid, $HOOC(CH_2)_7COOH$, and hence believe that it also contains an ester of an $R \cdot CH:CH(CH_2)_7$-$COOH$ acid, which may be oleic.

Capsanthin, $C_{35}H_{50}O_3$, or $C_{36}H_{50}O_3$

This pigment of the paparika (*Capsicum annuum*) was separated by Zechmeister (19, 20) from the ripe fruit.

Zechmeister and Cholnoky (83), by the use of suitable solvents, extracted from the fruit hard red waxes which, when hydrolyzed by 10 per cent potassium hydroxide in methyl alcohol, gave capsanthin, a yellow dye, and various acids. From capsanthin, by the action of the acid chlorides and pyridine, a number of esters were prepared—the diacetate, dipalmitate, dioleate, etc. The capsanthin obtained by hydrolysis of its specially purified esters gave analytical figures which agreed better with $C_{35}H_{50}O_3$, or $C_{36}H_{50}O_3$, than with the $C_{34}H_{48}O_3$ formula previously assigned to this product.

The pigment of the Japanese red pepper is also capsanthin (84), contrary to the statement of Bilger (53).

Oxidized by potassium permanganate, it gives dimethylmalonic and α,α'-dimethylsuccinic acids, but no α,α'-dimethylglutaric acid. Karrer (58) believes that at least one ring is present like that found in carotene and xanthophyll. Perhaps both ends of the chain are cyclic, since it forms no acetone when oxidized by ozone. There seem to be five isoprene units in the molecule.

Taraxanthin, $C_{40}H_{56}O_4$

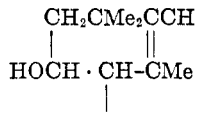
From dried dandelion (*Taraxacum officinale*) petals, Karrer and Salomon (41) isolated a xanthophyll identical with the one found by Schunck (4) in dandelion leaves.

Kuhn and Lederer (78), by extracting a large quantity of dried dandelion blossoms with organic solvents, saponifying the extract with alcoholic alkali, and purifying the saponified products, obtained a mixture of xanthophylls, from which they isolated taraxanthin (about 60 per cent of the total), $C_{40}H_{56}O_4$, as a new xanthophyll, also lutein, and a small amount of what appeared to be violaxanthin. Taraxanthin is isomeric with violaxanthin and resembles it closely. They probably possess the same carbon skeleton, with the hydroxyl groups in different locations.

Violaxanthin, $C_{40}H_{56}O_4$

This xanthophyll was extracted from the petals of the yellow pansy (*Viola tricolor*), where it occurs as an ester, by Kuhn and Winterstein (73), who made a careful study of it. It is also found as an ester in dandelion blossoms (78). The behavior of its perhydro derivative with methylmagnesium iodide indicates that its four oxygen atoms are all present as hydroxyl groups.

Karrer and Morf (68), on repeating this work, could detect only three hydroxyl groups by the Zerewitinoff method. The pure compound is a crystalline solid, m.p. 207–8° (corr.). Oxidized by potassium permanganate, it forms α, α'-dimethylsuccinic acid, like xanthophyll, zeaxanthin, and α-carotene, and hence probably also contains the ring



Fucoxanthin, $C_{40}H_{56}O_6$(?)

This pigment from the *Phaeophyceae*, or brown algae, was isolated by Willstätter and Page (13). Its molecular formula is probably $C_{40}H_{56}O_6$, and it possesses more strongly basic properties than many of the other carotenoids.

Hydrogenation experiments (58) in Karrer's laboratory indicate ten olefin bonds. Oxidized by potassium permanagnate, it yields acetic acid (4.5 moles) and dimethylmalonic acid, but no α, α'-dimethylsuccinic or α, α'-dimethylglutaric acids. It is related to carotene and to xanthophyll, and may contain two rings, both more highly hydroxylated than in the case of xanthophyll. In what form the six oxygen atoms are present, is still a puzzle.

Crocetins

These dyes of the saffron (*Crocus sativus*) have been the subject of some very interesting studies by Karrer and his coworkers, who have shown that there are three of them, α-, β- and γ-crocetin, and that the α-form occurs also (33) in the *Gardenia grandiflora*.

Karrer and Salomon (18, 23, 24) came to the conclusion that the α-crocetin was a dicarboxylic acid, $C_{17}H_{20}(COOH)_2$, of which β-crocetin was the monomethyl ester and γ-crocetin the dimethyl ester.

α-Crocetin adds seven moles of hydrogen and must therefore be acyclic ($C_{17}H_{34}(COOH)_2 = C_nH_{2n}(COOH)_2$). Its deep color, strong light absorption in the ultraviolet, intense halochromism with concentrated acids, and its many analogies with the diphenyl polyenes, $C_6H_5(CH:CH)_xC_6H_5$, of Kuhn and Winterstein, led to the conclusion that all its double bonds were conjugated.

The molecular refraction of dihydro-α-crocetin (crocetin itself could not be melted without decomposition) showed the presence of six double bonds in conjugation. Hence, α-crocetin itself contains seven such double bonds. Addition of the seven moles of hydrogen saturated these seven olefin bonds and, with the two carbons of the carboxyl groups, accounted for sixteen of the nineteen carbon atoms in the molecule. Oxidation of crocetin with potassium permanganate gave more than two moles of acetic acid. Assuming that the three remaining carbon atoms are present as methyl side chains, arranged as usual in terpene chains, the formula of α-crocetin would be as follows.

$$\underset{\alpha\text{-Crocetin}}{HOOCCH:(CHCMe:CHCH:)_3CHCOOH} = C_{19}H_{22}O_4$$

By addition of hydrogen to the double bonds, the color of α-crocetin is gradually discharged, α-crocetin being orange, the dihydro derivative (by titanium trichloride) sulfur yellow, and the hexahydro derivative nearly colorless. Its various hydrogenation products also show different stability to light.

The latest work on the saffron pigment (76), however, favors a $C_{20}H_{24}O_4$ formula, rather than $C_{19}H_{22}O_4$, for α-crocetin. This would make its perhydro reduction product, tetradecahydrocrocetin, $C_{20}H_{38}O_4$. The corresponding saturated hydrocarbon, crocetane (39), prepared by reduction of γ-crocetin (α-crocetin dimethyl ester) to the corresponding glycol, conversion of the latter to the dibromide and reduction of this dibromide, is therefore a $C_{20}H_{42}$, rather than a $C_{19}H_{40}$, and is perhaps a stereoisomer of phytane.

Crocin, the form in which the pigment occurs in the plant, is then $C_{18}H_{22}(COOC_{12}H_{21}O_{11})_2$, where the sugar groups are gentiobiose residues, and the formula for crocetin itself is probably as follows.

$$HOOCCMe{:}(CHCH{:}CHCMe{:})_3CHCOOH = C_{20}H_{24}O_4$$
α-Crocetin

This may be regarded as derived from norbixin by oxidative cleavage of two carbon atoms from each end of the chain. The methyl of the HOOC·CMe: group is assumed to be oxidized by potassium permanganate to pyruvic and not to acetic acid.

Bixin, $C_{25}H_{30}O_4 = HOOCC_{22}H_{26}COOMe$

Bixin, the pigment present in the seeds of the annatto tree (*Bixa orellana*) was reported first by Bolley and Piccard (2) and since has been studied by many chemists.

Recent investigations (34) have shown that it is the monomethyl ester of a dicarboxylic acid, resembling in this respect the β-crocetin of the saffron, that it contains nine olefin bonds (nine moles of hydrogen can be added catalytically), that it yields β-acetylacrylic ester by ozonolysis, *m*-xylene (26) on distillation, and four moles of acetic acid when oxidized by potassium permanganate. Further, it exists in stereoisomeric forms. On the basis of these and other experimental observations, Kuhn (26) proposed the following formula.

$$MeOOCCH{:}CHCMe{:}CHCH{:}(CHCMe{:}CHCH)_3CHCOOH$$
Bixin

Norbixin, the corresponding free dicarbonic acid, prepared from bixin by alkaline hydrolysis, like α-crocetin, gives with titanium trichloride both di- and hexa-hydro derivatives, whose properties resemble those of the crocetin derivatives, norbixin being brown-red, the dihydro derivative golden yellow, and the hexahydro derivative pale yellow. Their behavior toward light is similar to that of the corresponding α-crocetin derivatives.

Karrer, Stoll, and Stevens (69) have made use of bixin for the synthesis of higher hydrocarbons carrying numerous methyl side chains, of which group the highest synthesized theretofore was the perhydrolycopene (lycopane), $C_{40}H_{82}$, prepared from dihy-

drophytyl bromide and potassium, as noted in the discussion of lycopene.

Electrolysis of the potassium salt of perhydrobixin, $MeOCOC_{22}H_{44}COOK$, yielded the ester

$$MeOCOC_{22}H_{44}\cdot C_{22}H_{44}COOMe,$$

which was reduced to the corresponding glycol, then to the dibromide, which was in turn reduced to the hydrocarbon, $CH_3C_{22}H_{44}C_{22}H_{44}CH_3$, or $C_{46}H_{94}$ (dibixane). Assuming the structure proposed above for bixin, that for this dibixane would be

$$[Me(CH_2)_2(CHMeCH_2CH_2CH_2)_4-]_2$$

The neutral ester, $MeOCOC_{22}H_{44}C_{22}H_{44}COOMe$, was then partially saponified to the potassium salt of the ester acid, $MeOCOC_{22}H_{44}\cdot C_{22}H_{44}COOK$, electrolysis of which gave the ester $MeOCO(C_{22}H_{44})_4COOMe$, which with methylmagnesium iodide gave $Me_2C(OH)(C_{22}H_{44})_4C(OH)Me_2$. The latter was then reduced to $Me_2CH(C_{22}H_{44})_4CHMe_2$, or 2, 5, 9, 13, 17, 24, 28, 32, 36, 41, 45, 49, 53, 60, 64, 68, 72, 75-octadecamethylhexaheptacontane, $C_{94}H_{190}$, a viscous liquid which was not obtained perfectly pure.

Azafrin, $C_{28}H_{40}O_4$

A natural yellow pigment discovered by Liebermann (9, 12, 14) in azafranillo roots, and known as azafrin, has been investigated by Kuhn, Winterstein, and Roth (49), who have established its molecular formula as $C_{28}H_{40}O_4$, and have found that it adds seven moles of hydrogen to form perhydroazafrin, $C_{28}H_{54}O_4$, and that both are *dihydroxy monobasic acids* and optically active. The carboxyl group is readily esterified, but the hydroxyl groups could not be acylated and hence are believed to be tertiary. Oxidized by chromic oxide, azafrin gives an amount of acetic acid corresponding to five isoprene residues. In many ways it seems closely related to bixin and crocetin. They propose a formula,

$$C_{10}H_7(OH)_2(CH:CHCMe:CH)_3CH:CHCOOH$$

in which the $C_{10}H_7$ represents a residue composed of two isoprene units.

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