[CONTRIBUTION FROM THE WEIZMANN INSTITUTE OF SCIENCES]

ABSORPTION SPECTRA OF CAROTENOIDS; STRUCTURE OF VITAMIN A_2

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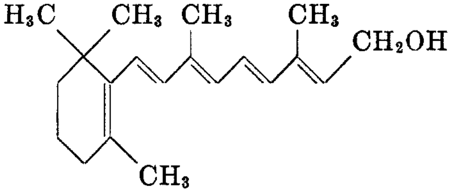
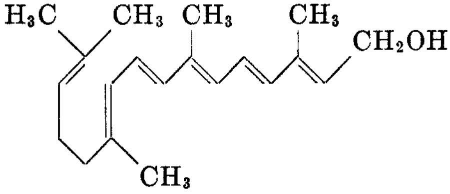
The ultraviolet absorption maxima of heteroannular dienes and trienes of the steroid series can be calculated with an accuracy of a few mμ from equation 1, in which m is the number of alkyl groups or ring residues attached to the polyene

$$1.\ \lambda_{\max} = 214 + 5m + 30\,(n - 2) + 5e$$

system, n is the number of conjugated double bonds, and e is the number of exocyclic double bonds (1, 2). A solvent effect, if any, is minor. For the pentaene alcohol vitamin A_1 the maximum calculated from the same equation (334 mμ) is in the order of magnitude of that found (326 mμ) but somewhat higher.



I Vitamin A_1



II

For vitamin A_2, however, the value 329 mμ calculated for formula II, proposed by Karrer, Geiger, and Bretscher (3) and favored by Shantz (4), is so divergent from the observed maximum of 351 mμ as to suggest that the formula may be incorrect. This discrepancy between observed and calculated absorption maximum prompted the following analysis of spectrographic data for carotenoids, conducted in the hope that an accurate method of calculation specific to these multiply unsaturated compounds might afford a basis for deduction of the actual structure.

Polyene systems. A typical carotenoid containing a polyene system of ten or eleven double bonds has a three-banded absorption spectrum in which the band of greatest intensity occupies a position almost exactly half-way between the other two and which thus represents the peak of a broad band differing from the single bands of dienes and trienes only in having fine detail in the form of shoulders that are usually developed into well-defined companion bands of shorter and longer wave length. The present analysis will thus be based solely upon the position of the maxima of the median bands of highest extinction coefficient.

The position of the absorption maxima of typical carotenoids is influenced to a considerable degree by the nature of the solvent, and it appears that sensitivity to solvent effect increases with increasing number of conjugated double bonds.

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Whereas solvents exert little effect on the absorption characteristics of steroid dienes (1), maxima recorded (5) for dehydrolycopene, which has fifteen conjugated double bonds, are as follows (mμ): hexane, 504; chloroform, 528, benzene, 531; pyridine, 535; carbon disulfide, 557. Wald (6) has noted that the spectra of carotenoid polyenes are very nearly the same in pentane, hexane, and ethanol, which have comparable refractive indexes, but are considerably displaced when the solvent is chloroform. Even greater displacements are observed in other solvents, particularly carbon disulfide. Thus, measurements in an aliphatic hydrocarbon or ethanol would seem the most suitable basis for comparison, but only a few results are available for ethanol solution. Maxima determined for hexane solutions are usually available and are taken as the basis for the present analysis; where data for hexane solutions are lacking, determinations made in petroleum ether, ligroin, or pentane are taken as equivalent to those in hexane.

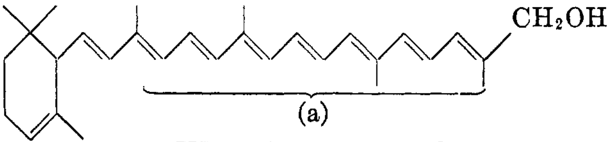
TABLE I

Maxima Calculated from Equation 1

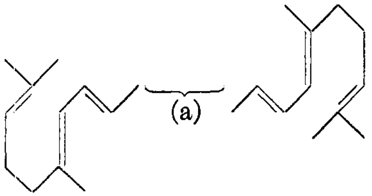
COMPOUND	n	m	$\lambda_{\max}^{\text{Hexane}}$, mμ	
			Calc'd	Found
α-Apo-2-carotenol	8	6	424	420
Lycopene type[a]	11	8	524	474 (av.)
Dehydrolycopene	15	10	654	504

[a] Lycopene, lycoxanthin, and lycophyll (see Table II).

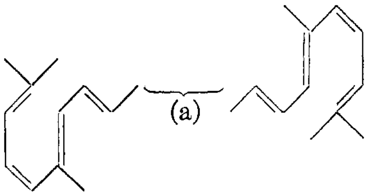
The inadequacy of equation 1 as applied to higher polyenes is demonstrated by the comparison given in Table I of maxima calculated and found (5) for the open-chain substances α-apo-2-carotenol (III), lycopene (IV), and dehydrolycopene (V), which contain, respectively, 8, 11, and 15 conjugated double bonds.



III α-Apo-2-carotenol



IV Lycopene



V Dehydrolycopene

The comparison suggests that the bathochromic effect of an additional double bond is not a constant quantity as assumed in Equation 1 but decreases with increasing number of conjugated double bonds.[2] As an approximation, the relationship can be described as a proportionality as expressed in equation 2, in which m is the number of alkyl substituents, n is the number of double bonds in conjugation, and A, x, and y are constants for the series. Substitution in 2 of the

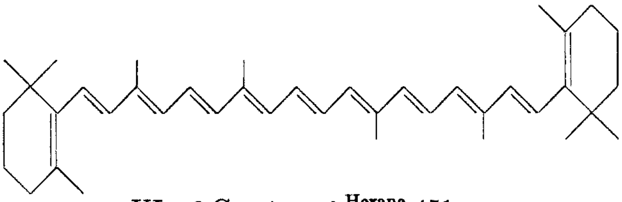
$$2.\ \lambda_{\max} = A + 5m + n\,(x - ny)$$

maxima and values for m and n of the polyenes III, IV, and V and of the arbitrarily assumed quantity $A = 114$ gives three simultaneous equations that yield the values $y = 1.80$, 1.61, and 1.69. From the average, $y = 1.7$, values found for x are 48.10, 47.79, and 48.16; av. $x = 48.01$. Hence equation 3 expresses the data. The series constant A was so selected that solution of the equation for

$$3.\ \lambda^{\text{Hexane}}_{\max} = 114 + 5\,m + n\,(48.0 + 1.7\,n)$$

$n = 1$ and for $n = 2$ gives the values 160 and 203 mμ, which are reasonably close to the maxima of ethylene and butadiene, respectively. The maxima calculated for III, IV, and V are 419, 476 and 501.5 mμ.

The bicyclic β-carotene (VI) contains the same number of conjugated double bonds as lycopene (eleven) but the chromophoric system has ten C-substituents as compared with eight for lycopene. If no other factor were involved, β-carotene should absorb at a wave length 10 mμ longer than lycopene, but actually the median maximum is 23 mμ less than that of lycopene. The hypsochromic effect evidently involved most likely is steric; possibly ring closure results in hindrance between the ring methyl groups and the side chain that prevents coplanarity and hence maximum resonance. γ-Carotene ($\lambda^{\text{Hexane}}_{\max}$ 462 mμ) is intermediate in structure, for one end of the molecule resembles lycopene and the other end resembles β-carotene. Since γ-carotene contains one more alkyl substituent than lycopene, the steric effect of ring closure (S) can be evaluated as follows:



VI β-Carotene, $\lambda^{\text{Hexane}}_{\max}$ 451mμ

$$S = \lambda^{\gamma\text{-Carotene}} - \lambda^{\text{Lycopene}} - 5 = -17$$

Comparison of γ- and β-carotene indicates the value $S = -16$. In these hydrocarbons with eleven double bonds the average effect of the presence of a ring characterized as having an internal double bond (R_i) is -16.5 mμ. Thus equation 3 can be modified by addition of the term $-16.5\ R_i$, where R_i is the number of rings of the type defined. Thus for vitamin A_1 ($m = 6$, $n = 5$, $R_i = 1$) the maximum is calculated as $114 + 30 + 197.5 - 16.5 = 325$ (found, 326).

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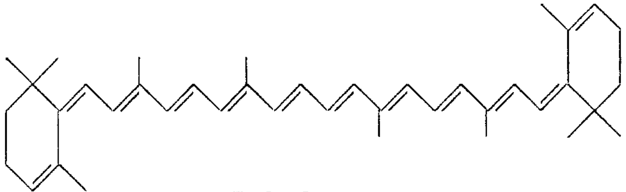
[2] Described by Brooker, Keyes, and Williams (7) as a convergent series; see also Kuhn (8).

Dehydro-β-carotene (VII) contains a second type of ring structure characterized by the presence of an exocyclic double bond (designation: R_e).



VII Dehydro-β-carotene

The exocyclic double bond *per se* may produce a bathochromic shift, and ring closure may be attended with a steric effect operating in the opposite direction. The net effect per ring as estimated from the value of $\lambda^{\text{Hexane}}_{\text{max}}$ found for dehydro-β-carotene (475 mμ) and that calculated from equation 3 (495 mμ) is 10 mμ.

All the above inferences are summarized in the following general equation for calculation of absorption maxima of carotenoid polyenes and of such oxygenated derivatives as carry no carbonyl groups in conjugation with the polyene system.

$$4.\ \lambda^{\text{Hexane}}_{\text{max}} = 114 + 5m + n\,(48.0 - 1.7\,n) - 16.5\,R_i - 10\,R_e$$

[m = no. of C-substituents; n = no. of conjugated double bonds; R_i = no. of rings with an internal double bond (type: VI); R_e = no. of rings with an external double bond (type: VII)].

A comparison of maxima for nineteen carotenoids calculated from equation 4 with those recorded in the compilation of Karrer and Jucker (5), is given in Table II. The agreement is good except in the case of β-apo-2-carotenol, and the reported maximum thus appears in doubt. The substances are probably of all-*trans* configuration except 5,6-dihydro-α-carotene, which is partly *cisoid*. The table includes computations pertaining to the solvent effect. Little or no displacement of the spectrum occurs in ethanol solution as compared with hexane solution, but displacements of increasing magnitude are noted in chloroform, benzene, pyridine, and carbon disulfide. For α-apo-2-carotenol, with eight double bonds, the displacement between carbon disulfide and hexane solution is 25 mμ; for dehydrolycopene, with fifteen double bonds, the displacement is 53 mμ. The suggestion that the bathochromic solvent effect is proportional to the number of conjugated double bonds was tested by computation of the quantity $c = (\lambda^{\text{Solvent}} - \lambda^{\text{Hexane}})/n$, and the results tabulated indicate that this quantity is indeed a constant for a given solvent. The average values found for c may be used with fair assurance for calculation of λ_{max} in one solvent from the value found for another solvent. The dependence of the solvent effect upon n explains why no effect has been apparent among steroid dienes.

Aldehydes, ketones, acids. The α,β-unsaturated carbonyl compounds of the carotenoid series seem to differ considerably in absorption characteristics from steroid enones and dienones; the relationships appear more comparable with those noted among corresponding polyenes. Thus compounds VIII and IX,

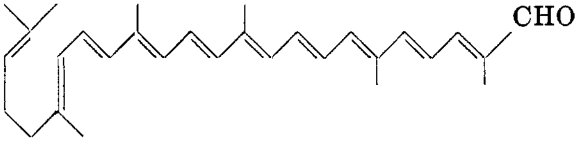
TABLE II

ABSORPTION MAXIMA FOR CAROTENOID POLYENES AND DERIVATIVES CALCULATED FROM EQUATION 4

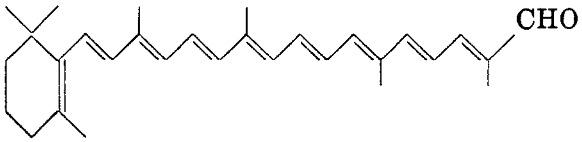
COMPOUND	n	m	RINGS		$c = \frac{\lambda\text{ Solvent} - \lambda\text{ Hexane}}{n}$					$\lambda_{\max}^{\text{Hexane}}$	
			R_i	R_e	EtOH	Chf	C_6H_6	Py	CS_2	Calc'd	Found
α-Apo-2-carotenol	8	6	–	–	0.4				4.0	419	420
5,6-Dihydro-α-carotene	9	6	–	–	0.05	1.1	2.2		4.9	438	442.5
Lycopene	11	8	–	–	−0.3	0.4	1.1		2.7	476	475.5
Lycoxanthin	11	8	–	–	0.09		1.3		3.0	476	473
Lycophyll	11	8	–	–	0.09		1.3		3.0	476	473
Dehydrolycopene	15	10	–	–		1.6	1.7	2.1	3.6	501.5	504
Vitamin A_1	5	6	1	–	0					325	326
β-Apo-2-carotenol	9	8	1	–	0.3				3.7	432	423
α-Carotene	10	8	1	–		0.7			3.0	447.5	447.5
5,6-Dihydro-β-carotene	10	8	1	–	0.05	1.0	1.1		2.9	447.5	447.5
Xanthophyll	10	8	1	–	−0.1	0.9			2.8	447.5	447.5
Capsanthol	10	8	1	–		0.8	1.4	1.5	2.9	447.5	448[a]
γ-Carotene	11	9	1	–		1.2	1.4		3.1	465	462
Rubixanthin	11	9	1	–	0.1	1.1			2.9	465	462
β-Carotene	11	10	2	–		1.4			3.1	453	451
Cryptoxanthin	11	10	2	–		1.1			2.9	453	451
Zeaxanthin	11	10	2	–	−0.05	1.0			2.8	453	451.5
Dihydrorhodoxanthin	11	10	2	–	−0.2	0.7			2.5	453	452
Dehydro-β-carotene	12	10	–	2		0.8			2.4	475	475
Averages					c = 0.03	1.0	1.4	1.8	3.1		

[a] Taken as equal to $\lambda_{\max}^{\text{EtOH}}$.

with nine double bonds conjugated with a carbonyl group, have maxima ($\lambda_{\max}^{\text{Hexane}}$ 473, 454 mμ) not far from those calculated for structurally related polyenes with ten conjugated ethylenic linkages (454, 442.5 mμ). Computation from available



VIII Apo-3-lycopenal



IX β-Apo-2-carotenal

data by the methods developed above has led to the empirical equations 5, 6, and 7 given below, and calculated maxima are compared with those found in Table IV. Data are available for open-chain aldehydes having 8, 9, and 10 conjutaged ethylenic linkages (Table III: α-citraurin and α-apo-2-carotenal, apo-3-lycopinal, and apo-2-lycopenal), and solution of simultaneous equations as before affords the values of x and y given in equation 5. Unfortunately evidence regarding the effect of a ring with an internal double bond (R_i) is conflicting. In the case of retinene$_1$, or vitamin A_1 aldehyde, the effect of such a ring appears to be negligible; in the case of β-apo-2-carotenal (IX) and its hydroxy derivative β-citraurin, the effect is 20–24 mμ. No reason for this discrepancy is apparent; equation 5 includes a term ($-10\ R_i$) that averages the two divergent estimates.

The data in this and related series are very meagre as well as partly conflicting, but the following tentative conclusions seem indicated. Aldehydes have the same absorption characteristics as the corresponding acids, and esters are equivalent to acids. In a dialdehyde or diacid the second functional group is counted merely as a carbon-substituent, equivalent to an alkyl group, and the equation for the monofunctional compounds is then applicable. Ketones are slightly less powerful chromophores than aldehydes and acids. In diketones, unlike dialdehydes and diacids, the second carbonyl group has an appreciable bathochromic effect. The diketone system is treated as a unit and the second carbonyl group is not counted as an alkyl substituent; terminal groups attached to the system —CO(CH═CH)$_n$ CO— are not counted as alkyl substituents. In some instances the agreement with reliable data is only very approximate (retinene$_1$); in other instances the data are incomplete or uncertain (parentheses). Where the only measurements available are in a solvent other than hexane or ligroin (last column), the solvent concerned is indicated. The effect of a ring with an external double bond (R_e), as estimated from a single instance (rhodoxanthin), appears to be twice as great as in the polyene series.

Aldehydes, dialdehydes, acids, diacids

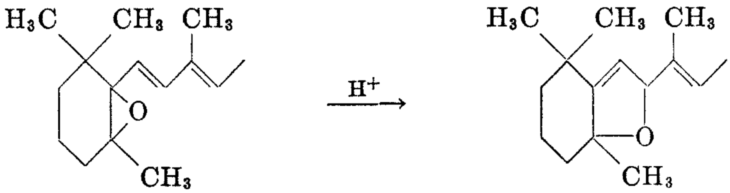
$$5.\ \lambda^{\text{Hexane}}_{\text{max}} = 114 + 5m + n\,(55.5 - 2.1\,n) - 10\ R_i$$

Ketones:

$$6.\ \lambda^{\text{Hexane}}_{\text{max}} = 100 + 5m + n\,(55.5 - 2.1n) - 10\ R_i$$

Diketones:

$$7.\ \lambda^{\text{Hexane}}_{\text{max}} = 120 + 5m + n\,(55.5 - 2.1n) - 10\ R_i - 18\ R_e$$



X XI

TABLE III

ALDEHYDES, KETONES, ACIDS

COMPOUND	n	m	RINGS		$c = \frac{\lambda\text{ Solvent} - \lambda\text{ Hexane}}{n}$				$\lambda_{\text{max}}^{\text{Hexane}}$	
			R_i	R_e	EtOH	Chf	C_6H_6	CS_2	Calc'd	Found
ALDEHYDES AND DIALDEHYDES (EQUATION 5)										
Retinene$_1$^{a}	5	5	1		3.0	4.4			354	365
Apo-1-azafrinal	6	4						5.0	391	(431^b)
α-Citraurin	8	5						5.0	449	450
α-Apo-2-carotenal	8	5						4.2	449	450
Apo-3-lycopenal	9	6					1.7	4.4	473	473
β-Apo-2-carotenal	9	7	1					4.0	468	454
β-Citraurin	9	7	1				1.0	3.6	468	458
Apo-2-lycopenal	10	6						3.8	489	490.5
Apo-3,12-lycopenedial	8	5						4.0	449	452
Apo-2,12-lycopenedial	9	5				2.5		3.8	468	468
ACIDS,^{c} DIACIDS,^{c} ALDEHYDE-ACIDS (EQUATION 5)										
Vitamin A acid	5	5	1						354	EtOH 351.5^d
Azafrin	7	4				1.1			420	422.5
3,8-Dimethyldecapentaene-1,10-dicarboxylic acid	5	3							CS_2 375^e	CS_2 (419^b)
Crocetin	7	5				1.4		4.1	425	424.5
Bixin	9	5							Chf 481^e	Chf 475
Apo-2-norbixinal	7	4						4.1	420	424
Apo-1-norbixinal	8	5				1.4		4.1	449	445
KETONES (EQUATION 6)										
Myxoxanthophyll (?)	10	6							475	EtOH 471
Capsanthin	10	7	1				1.1	2.8	470	475
Semi-β-carotenone	10	6	1				1.7	3.0	465	469
Myxoxanthin (?)	11	8	1		0.5	0.7		2.1	486	(465^b)
Anhydrosemi-β-carotene	11	9	1			0.8	0.9	2.6	491	480
DIKETONES, KETOALDEHYDES (EQUATION 7)										
β-Carotenone	9	4				2.6	2.2	3.5	469	466
Physalienone	9	4				2.7		4.0	469	464
Capsanthinone	9	4					1.7	3.4	469	472
Capsorubin	9	4					1.3	3.2	469	474
Anhydrocapsanthinone	10	6	1				1.6	3.4	485	483
Astaxanthin	11	8	2						496	Py 493
Bisanhydro-β-carotenone	11	8	2						496	494
Rhodoxanthin	12	8		2	0.7	1.8	1.3	3.1	488	488
β-Carotenonaldehyde	7	3				2.7	2.1	3.9	421	431
4-Hydroxy-β-carotenonaldehyde	7	3					2.3	3.8	421	433

[a] Constants from Wald (6). [b] Only one band reported. [c] Esters and acids have identical absorption characteristics. [d] See Wendler, Slater, and Tishler (9). [e] Calculated from [c] for crocetin.

Oxides. Carotenoid epoxides (X) and the furanoid oxides (XI) into which they are converted by acids are listed in Table IV. It appears that an oxide linkage adjacent to the conjugated system has a slight bathochromic effect and that a ring with an internal double bond produces a slightly greater hypsochromic shift than in the polyenes. The empirical equation 8 used for calculation is a slight modification of the polyene equation 4.

$$8.\ \lambda^{\text{Hexane}}_{\text{max}} = 118 + 5m + n\,(48.0 - 1.7\,n) - 23\ R_i$$

TABLE IV

OXIDES (EQUATION 8)

COMPOUND	n	m	R_i	$c = \frac{\lambda\ \text{Solvent} - \lambda\ \text{Hexane}}{n}$					$\lambda^{\text{Hexane}}_{\text{max}}$	
				EtOH	Chf	C_6H_6	Py	CS_2	Calc'd	Found
EPOXIDES AND DIEPOXIDES										
α-Carotene monoepoxide	9	6			1.3	1.4		3.2	442	442
Xanthophyll epoxide	9	6		0.3		1.2		3.3	442	442
β-Carotene monoepoxide	10	8	1		1.2	1.3		3.2	445	447
Cryptoxanthin epoxide	10	8	1						445	EtOH 449
Antheroxanthin	10	8	1						445	Chf 460.5
β-Carotene diepoxide	9	6			1.4	1.4		3.2	442	443
Cryptoxanthin diepoxide	9	6							442	EtOH 442
Violaxanthin	9	6		−0.1	1.0			3.0	442	443
FURANOID OXIDES AND DIOXIDES										
Flavochrome	8	6			1.4	1.5		3.6	423	422
Flavoxanthin	8	6		0	1.1			3.5	423	421
Chryanthemoxanthin	8	6		0	1.1			3.5	423	421
Mutatochrome = Citroxanthin	9	8	1		1.2	1.4		3.5	429	427
Cryptoflavin	9	8	1						429	EtOH 430
Mutatoxanthin	9	8	1	0.1	1.1	1.4	1.9	3.7	429	426
Cryptochrome	7	6							401	CS_2 424
Aurochrome	7	6							401	CS_2 428

Solvent effect. The average coefficients (c) relating absorption maxima in various solvents to the maxima in hexane are summarized in Table V. Only in the series of polyene carotenoids is there a sufficient body of data for comparison of absorption characteristics in hexane, ethanol, chloroform, benzene, and carbon disulfide. The concordance of the maxima in ethanol and in hexane and the progressive bathochromic shifts that occur in the other three solvents appear to bear some relationship to the refractive indexes. Wald (6) suggested that the large displacements in the spectra of retinene$_1$ and rhodoxanthin between hexane and ethanol or chloroform represent a specific characteristic of carotenoids which contain a conjugated carbonyl group. According to the present analysis the displacements are abnormal only in the case of retinene$_1$; rhodoxanthin contains twelve ethylenic linkages, and the displacement per double bond is about the same as in the polyene series. The available data suggest that ketones, diketones, and oxides are comparable with respect to solvent effect to polyenes and that the spectra of aldehydes and acids are subject to somewhat greater displacements.

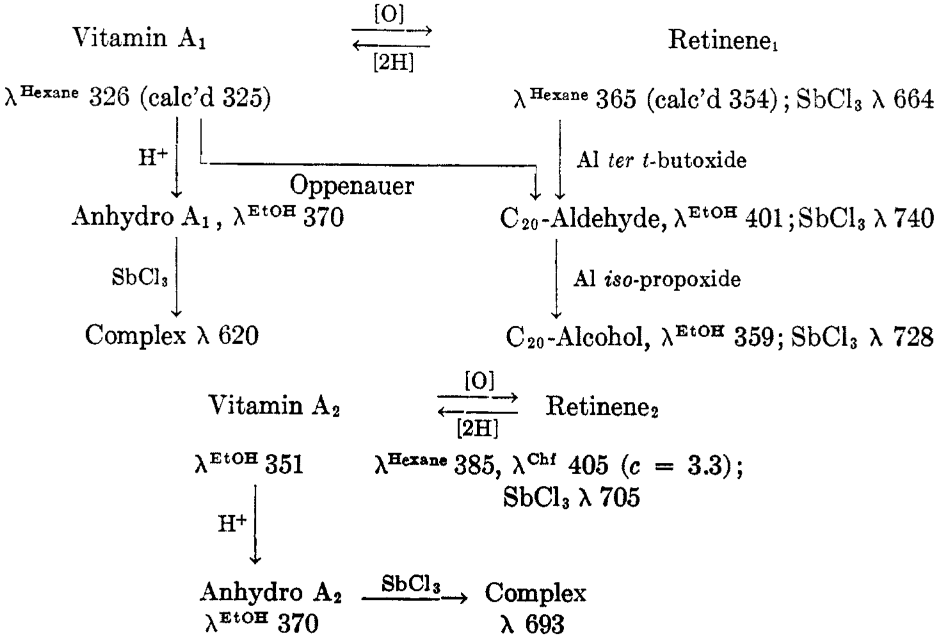
Vitamin A_2. The observations concerning vitamins A_1 and A_2 requiring interpretation in terms of structural formulas are summarized in the following chart of transformations, which gives the maxima (in mμ) of the sole or median absorption bands of the derivatives and of the antimony trichloride complexes.

TABLE V

SOLVENT EFFECT

COMPOUND TYPE	COEFFICIENTS (c) FOR SOLVENTS (n_D^{20} IN PARENTHESES)					
	Hexane (1.357)	EtOH (1.361)	Chf (1.446)	C_6H_6 (1.502)	Py (1.501)	CS_2 (1.628)
Polyene systems	0	0.03 (12[a])	1.0 (14[a])	1.4 (8[a])	1.8 (2[a])	3.1 (18[a])
Aldehydes, dialdehydes	0	3.0 (1)	3.4 (2)	1.3 (2)		4.2 (9)
Acids, diacids, aldehyde-acids	0	1.4 (1)	1.2 (2)			4.1 (3)
Ketones	0	0.5 (1)	0.7 (2)	1.8 (3)		2.8 (3)
Diketones, ketoaldehydes	0	0.7 (1)	2.5 (4)	1.8 (7)		3.5 (8)
Oxides (1,2- and 1,4-)	0	0 (5)	1.2 (9)	1.4 (7)	1.9 (1)	3.4 (10)

[a] Number of comparisons.

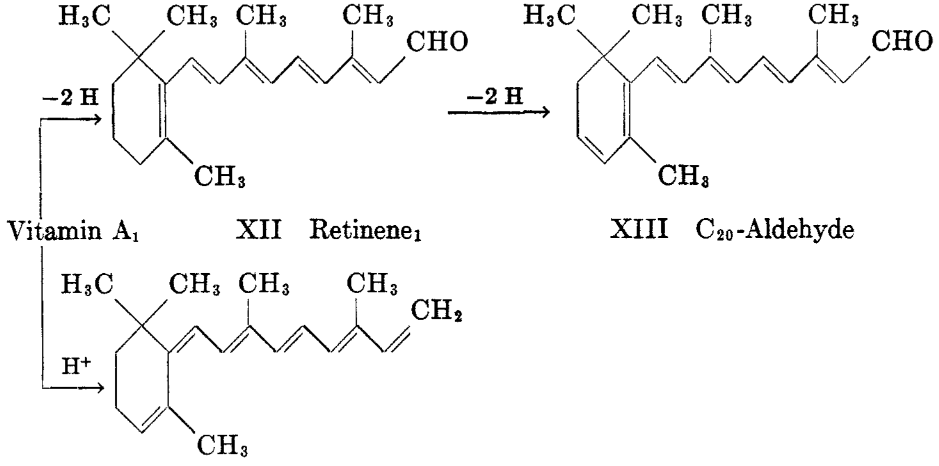


Vitamin A_1 is oxidized reversibly *in vivo* to the aldehyde retinene (10); oxidation can be accomplished by adsorption on manganese dioxide from petroleum ether

(11, 6), and reduction has been effected enzymatically *in vitro* (12). Vitamin A_1 is easily dehydrated to an anhydro compound; Oppenauer oxidation of the alcohol or aldehyde gives a C_{20}-aldehyde very similar in absorption characteristics to the aldehyde of vitamin A_2 and reducible to an alcohol similar to or identical with A_2 (13, 14). Vitamin A_2, isolated in pure form by Shantz (4), distills in a high vacuum at a temperature only about 3° higher than A_1 (15) and is so similar to A_1 in chromatographic characteristics and ease of dehydration that it must be an allylic alcohol of nearly the same molecular weight. From a comparison with the distillation characteristics (elimination maxima) of saturated and unsaturated fatty acids, Gray and Cawley (16) concluded that A_2 has the same number of carbon atoms as A_1 but contains one additional conjugated double bond. Although anhydro A_2 (4) contains seven double bonds whereas anhydro A_1 contains six, it has a three-banded spectrum with maxima corresponding exactly in position to those of anhydro A_2 and differing only in being somewhat less intense. The two anhydro compounds are differentiated, however, by the absorption maxima of the antimony trichloride complexes: 620 mμ for A_1 and 693 mμ for A_2 (17).

Chemical evidence of structure is incomplete and in part conflicting. Karrer found that ozonization of vitamin A_2 preparations containing very little A_1 gave some 8% of acetone (3) and 55–60% of formaldehyde (18). He pointed out that the result does not necessarily indicate a mixture of isomers having the groupings $(CH_3)_2C=$ and $CH_2=C(CH_3)—$ but may be the result of rearrangement induced by ozone; both lycopene and β-carotene were found to yield considerable formaldehyde on ozonization. Analytical evidence is not extensive. A single analysis reported by Shantz (4) for crystalline vitamin A_2 phenylazobenzoate is in good agreement with the open-chain formula $C_{20}H_{30}O$ for vitamin A_2 (II) proposed by Karrer, but also agrees satisfactorily with the formula $C_{20}H_{28}O$ for a cyclic structure. A single analysis of Shantz's anhydro A_2 agrees with the formula $C_{20}H_{28}$ but not with the formula $C_{20}H_{26}$, and a single analysis of the best preparation of A_2 in Karrer's laboratory is in fair agreement with the formula $C_{20}H_{30}O$ and does not check with the formula $C_{20}H_{28}O$. Two conflicting conclusions based upon mixed melting point determinations have been reported. Karrer and Bretscher (18) found that the allophanate of vitamin A_2 did not depress the melting point (73°) of the allophanate of dihydrophytol and concluded that A_2 has the open-chain structure II. Morton, Salah, and Stubbs (14) found that the 2,4-dinitrophenylhydrazones of retinene$_2$ and the C_{20}-aldehyde from vitamin A_1 melt at the same temperature and show no depression on admixture, and they concluded that A_2 has a cyclic structure. It thus appears that ozonization probably follows an anomalous course, that analytical evidence favors an open-chain structure but is not conclusive, and that in this series closely related compounds may or may not exhibit easily recognized melting point depressions.

Even in the A_1 series the structures cannot be deduced unambiguously from existing spectrographic data for the carotenoids because these data include certain inconsistencies, as noted above, and because there is no basis for estimation of the chromophoric power of certain possible structural types. Retinene$_1$ ($n = 5$) can hardly have any structure other than XII and yet the maximum



Vitamin A$_1$ XII Retinene$_1$ XIII C$_{20}$-Aldehyde

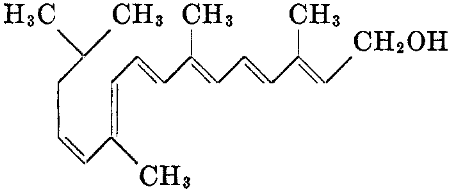
XIV Anhydro A$_1$

at 365 mμ is 24 mμ higher than expected in analogy with β-apo-2-carotenal (IX, $n = 9$). However the maximum found for retinene$_1$ is surely the better basis for comparison with the value 401 mμ reported for the C$_{20}$-aldehyde obtained by Oppenauer oxidation of either A$_1$ or retinene$_1$. The bathochromic shift suggests that the conjugated system has been lengthened. Oppenauer oxidation of pregnenolone under certain conditions has been shown to be attended with introduction of an additional double bond (19), and hence the C$_{20}$-aldehyde may be dehydroretinene$_1$, XIII, as suggested by Haworth, *et al.* (13). The maximum calculated for XIII from equation 5 on the assumption that the R$_i$ effect is negligible, as in the case of retinene$_1$, is 396 mμ, in agreement with the observed maximum. There is no basis for judging whether or not the presence of two double bonds in the ring should produce a bathochromic displacement, and hence formula XIII tentatively satisfies the known requirements.

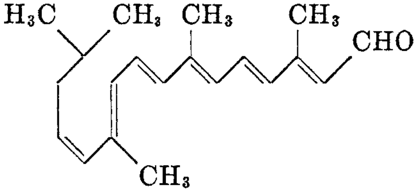
The expected product of dehydration of vitamin A$_1$ is the hexaene XIV, which has a ring with an external double bond. If the R$_e$ effect is the same as in the dodecaene dehydro-β-carotene, the maximum calculated for XIV (356 mμ) is considerably less than that found (370 mμ). If the R$_e$ effect is negligible in the hexaene, like the R$_i$ effect in retinene$_1$, XIV should have a maximum of 366 mμ, close to that found.

Since in the retina of certain fresh water fish vitamin A$_2$ and retinene$_2$ are components of a reversible oxido-reduction system (10), it seems necessary to accept the proposition that no bond migration or other abnormality is involved in the oxidation of A$_2$ to the aldehyde or in the reverse process of chemical or enzymatic reduction. This proposition means for one thing that, if vitamin A$_2$ is an allylic alcohol, the carbonyl group of retinene$_2$ must be conjugated with the polyene system. Experimental evidence that this is the case is found in the large displacement of the spectrum between hexane and chloroform ($c = 3.3$).

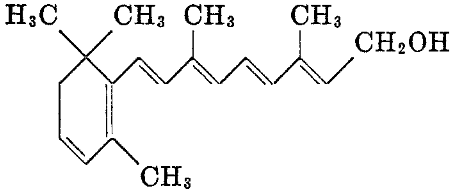
Vitamin A_2 contains one double bond more than vitamin A_1, and in the Karrer formula II this additional double bond is not conjugated with the other five. The absorption maximum calculated for II from equation 4 is 336 mμ, which is considerably lower than the value found (351 mμ). The maximum calculated from equation 5 for the corresponding aldehyde (359 mμ) is also wholly out of line with the maximum observed for retinene$_2$ (385 mμ). The evidence of Gray and Cawley (16) cited above indicates conjugation of the sixth double bond with the other five. One possibility is that the substance is the open-chain polyene alcohol XV and that retinene$_2$ is the aldehyde XVI. Reliable calculation of the maxima for these structures can be made from equations 4 and 5; that for the aldehyde agrees with the value found for retinene$_2$ but the maximum calculated for the alcohol XV (366 mμ) is incompatible with that found for vitamin A_2 (351 mμ), and hence the formulation must be rejected. Another possibility is that vitamin A_2 has the structure XVII, considered by Gillam, Heilbron, Jones, and Lederer (20) and favored by Gray (16). Retinene$_2$ would then have the formula XIII, above, which accounts adequately for the spectrum, and it would be identical with the C_{20}-aldehyde resulting from oxidation of retinene$_1$. Since the alcohol XVII represents the dehydro derivative of vitamin A_1, the hypsochromic R_i effect can be assumed to be operative as in A_1, and the maximum calculated from equation 4 (354.5 mμ) is close to that found (351 mμ). Karrer and Bretscher (18) rejected formula XVII because the substance yielded no α,α-dimethylsuccinic acid on ozonization, but since ozonization follows an anomalous course to the extent of yielding formaldehyde and acetone the validity of the negative evidence cited is open to some question. Another objection to the formula is that dehydration would be expected to proceed exactly as with A_1 by elimination of water across the conjugated system to give a heptaene, whereas anhydro A_2 appears to contain only a hexaene system.



XV, $\lambda_{\text{calc'd}}^{\text{Hexane}}$ 366 mμ

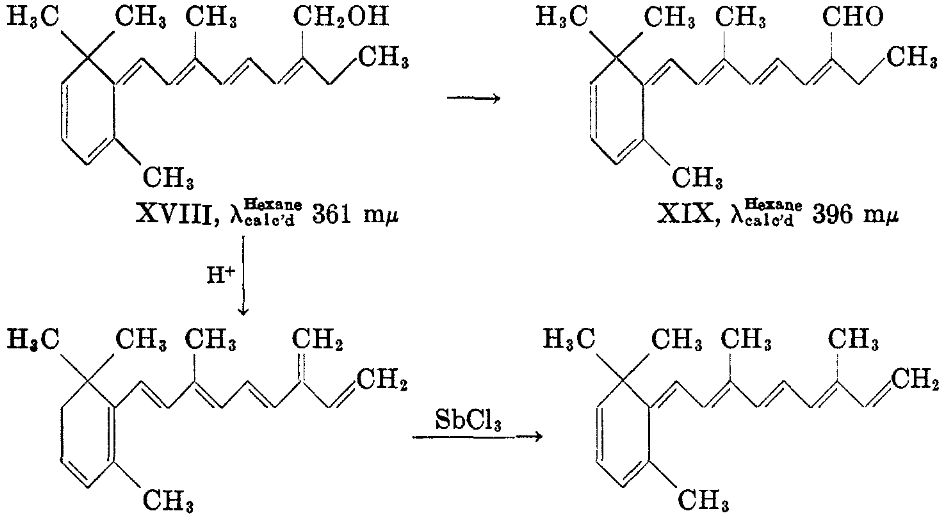


XVI, $\lambda_{\text{calc'd}}^{\text{Hexane}}$ 391 mμ



XVII, $\lambda_{\text{calc'd}}^{\text{Hexane}}$ 354.5 mμ

The alternate formula XVIII accounts for the absence of α,α-dimethylsuccinic acid from the products of ozonization and offers a possible explanation of the difference between A_2 and A_1 in their behavior on dehydration. In XVIII the carbon atom adjacent to the end of the polyenic alcoholic system carries no hydrogen atom and hence direct dehydration across the conjugated system is not possible. An alternate sequence of reactions might lead to an anhydro compound of structure XX. This structure has a cross-conjugated system and would be expected to have the absorption characteristics of a hexaene rather than a heptaene. Calculation presents the same uncertainties as in the case of formula XIV for anhydro A_1 and the conclusions are practically the same: the maxima expected for XX if the R_i effect is operative and non-operative, respectively, are 349.5 and 366 mμ (found, 370 mμ). In any case XIV and XX should have very similar absorption spectra and hence are possible representations of anhydro A_1 and anhydro A_2. The difference in the maxima of the antimony chloride complexes is also accounted for on the assumption that XX is isomerized by the reagent to XXI, which has a normal heptaene system.



XVIII, $\lambda_{\text{calc'd}}^{\text{Hexane}}$ 361 mμ

XIX, $\lambda_{\text{calc'd}}^{\text{Hexane}}$ 396 mμ

XX

XXI

The calculation of the maximum for XVIII is subject to the uncertainties discussed with reference to XVII and the value given (361 mμ) thus appears close enough to the actual value (351 mμ) to admit the formula as a possibility. A substance of the alternate structure XVII could yield the anhydro compound XX by 1,4-elimination of water across the terminal double bond, but it would then be difficult to see why A_2 should behave any differently from A_1. Formula XVIII for vitamin A_2 thus seems to offer a better general account of the properties of the alcohol and its transformation products than any of the alternate formulas. The interpretation suggested implies that retinene$_2$ (XIX) and the C_{20}-aldehyde for vitamin A_1 (XIII) have similar absorption characteristics but are not identical, in spite of the mixed melting point evidence of identity. It discounts also the mixed melting point evidence of an open-chain structure. Perhaps the most serious objection is that the cyclic formula is consistent with only one of three available analyses. A final decision thus awaits further evidence.

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Note added in proof. Since this paper was written, Karrer and Schneider, *Helv. Chim. Acta*, **33**, 38 (1950), have reported the preparation by the method of Shantz (4) of a sample of vitamin A_2 phenylazobenzoate melting somewhat higher than Shantz's sample. They report an analysis of the derivative that agrees better with the cyclic than with the open-chain formula and state that neither the derivative nor the vitamin A_2 obtained from it afforded any acetone on ozonization.

SUMMARY

Empirical equations have been developed for calculation of the absorption maxima of all types of natural carotenoid pigments; maxima calculated for over sixty-five compounds are in substantial agreement with the values found.

The magnitude of the displacements of the spectra of a series of compounds in different solvents is proportional to the number of conjugated double bonds.

Analysis of the spectrographic data regarding the properties and transformations of vitamins A_1 and A_2 has led to consideration of new formulas for vitamin A_2 and derived compounds.

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