503. *Studies in the Polyene Series. Part XLIII.* The Structure and Synthesis of Vitamin A_2 and Related Compounds.*

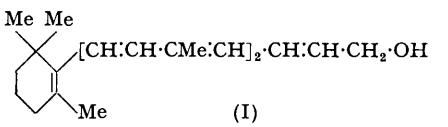
By (Mrs.) K. R. Farrar, J. C. Hamlet, H. B. Henbest, and E. R. H. Jones.

A dehydrovitamin A_1 (II) has been synthesized and shown to be identical with vitamin A_2. The aldehyde (retinene$_2$) and anhydrovitamin A_2, both crystalline solids, have been prepared from the synthetic vitamin. The ultra-violet and infra-red light absorption properties of these and related compounds with shorter side-chains are discussed. A preliminary account of this work has been published (*Chem. and Ind.*, 1951, 49).

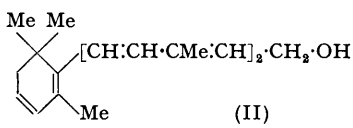
The presence in fish-liver oils of a compound closely related to vitamin A_1 was first indicated by light-absorption measurements by Edisbury, Morton, and Simpkins (*Nature*, 1937, **140**, 234) and Gillam, Heilbron, Lederer, and Rosanova (*ibid.*, p. 233). Certain oils or concentrates gave an antimony trichloride colour which exhibited maximal absorption at 6930 Å, the corresponding vitamin A_1 maximum being at 6200 Å. The new compound, which could not be separated from vitamin A_1, was termed vitamin A_2 by Morton and his co-workers. The ratio of the two vitamins present in fish oils varies according to species, but, in general, oils from freshwater fish contain more vitamin A_2 than A_1, the converse being true for marine fish (cf. Gillam, Heilbron, Jones, and Lederer, *Biochem. J.*, 1938, **32**, 405).

From these studies with vitamin A_2 concentrates it was apparent that the main light-absorption maximum of vitamin A_2 is located at a considerably longer wave-length than that of vitamin A_1 (3260 Å). Recently, Shantz (*Science*, 1948, **108**, 417) has obtained from the oil from selected pike livers a sample of the vitamin of high purity, which has a main absorption band at 3510 Å, and a well-defined subsidiary maximum at 2870 Å (see Fig. 1).

The difficulty of obtaining pure vitamin A_2 has hampered work on the elucidation of its structure, but nevertheless several formulæ have been suggested. Gillam, Heilbron, Jones, and Lederer (*loc. cit.*) noted that structure (I), a vinylogue of vitamin A_1, would account for the absorption of vitamin A_2 at longer wave-length. Gray and Cawley (*J. Biol. Chem.*, 1939, **131**, 317; 1940, **134**, 397) suggested a dehydrovitamin A_1 structure (II), after a study of the relative volatilities of the two vitamins had indicated their similar molecular weights; this formula has also been favoured by Morton, Salah, and Stubbs (*Nature*, 1947, **159**, 744). Karrer and his collaborators (*Helv. Chim. Acta*, 1943, **26**, 1758, and earlier references) put forward the open-chain structure (III), in the belief that the position of the main absorption maximum would be in better accord with this structure than with (II), and adduced support from the isolation of acetone in over 60% yield on ozonolysis of a vitamin A_2 concentrate. Karrer and Schneider (*ibid.*, 1950,



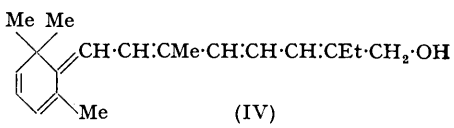
(I)



(II)

$Me_2C{:}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CMe{:}CH{\cdot}[CH{:}CH{\cdot}CMe{:}CH]_2{\cdot}CH_2{\cdot}OH$

(III)



(IV)

33, 38) later reported that a purer sample of vitamin A_2 did not yield acetone on ozonolysis. After an attempt to correlate the light-absorption data of carotenoids with their structures, Fieser (*J. Org. Chem.*, 1950, **15**, 930) proposed the rather more speculative structure (IV).

In view of the difficulty of obtaining sufficient quantities of pure vitamin A_2 for detailed structural studies, it seemed to us that the vitamin A_2 problem could best be solved by

* Part XLII, *J.*, 1952, 1414.

synthesis, especially since a variety of satisfactory procedures have been developed in connection with the synthesis of vitamin A_1 (for a summary see Heilbron, *J.*, 1948, 386). The dehydrovitamin A_1 structure (II) appeared to be the most plausible on various grounds, particularly since it affords an explanation of the second absorption band at 2870 Å (see later).

The first approach attempted was from 3 : 4-dehydro-β-ionone (V), following one of the now classical routes to the C_{20} alcohol devised in the A_1-series starting from β-ionone. The ketone, prepared in 40% yield from β-ionone by treatment with *N*-bromosuccinimide followed by dehydrobromination (Henbest, *J.*, 1951, 1074), was treated with methyl γ-bromocrotonate, giving a hydroxy-ester with light absorption in agreement with the expected structure (VI). However, dehydration with toluene-*p*-sulphonic acid (as in the preparation of the C_{17} acid from β-ionone) gave material with an absorption maximum at too short a wave-length for the expected ester (VII; R = Me) (see later) and hydrolysis

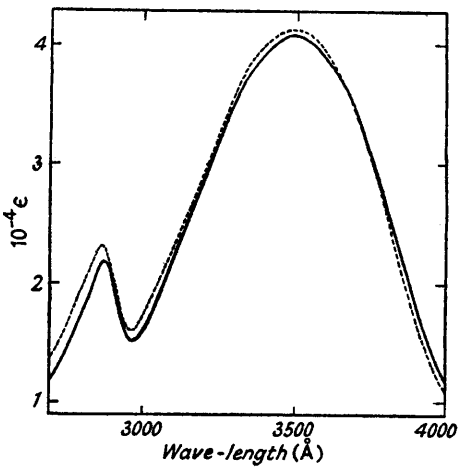
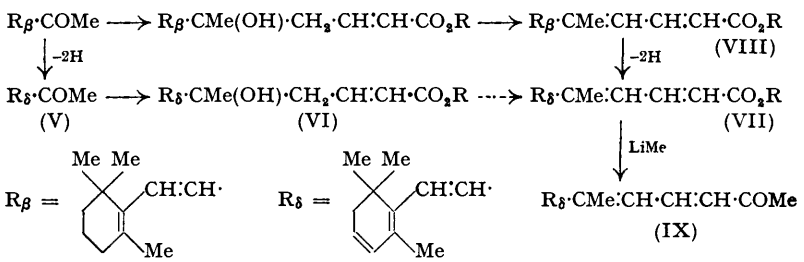


FIG. 1. *Ultra-violet absorption of vitamin A_2.*

------ *Natural compound.*
——— *Synthetic compound.*

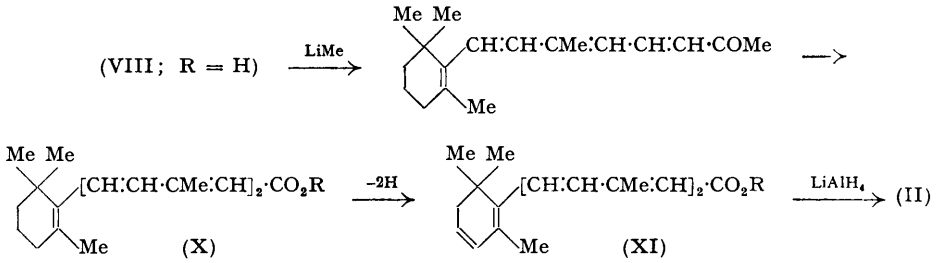
failed to yield a crystalline acid. It is possible that 1 : 8-dehydration (into the terminal ring) occurred, giving rise to a tetraene chromophore.

An alternative procedure involved the bromination and dehydrobromination of the well-known methyl ester (VIII; R = Me) of the C_{17} acid, and the building up of the side-chain *via* the C_{18} dehydro-ketone (IX). The ester reacted very much more readily than β-ionone with *N*-bromosuccinimide, bromination occurring at room temperature. Dehydrobromination was carried out with 4-phenylmorpholine, structurally related to diethylaniline, but more readily purified. Hydrolysis of the methyl ester gave the



crystalline C_{17} dehydro-acid (VII; R = H) in 55% overall yield, converted by methyl-lithium into the C_{18} dehydro-ketone (IX). Although a Reformatsky reaction between this ketone and methyl bromoacetate appeared to occur (the crude hydroxy-ester showing $\lambda_{max.}$ 3300 and 2700 Å), no crystalline C_{20} dehydro-acid (XI; R = H) could be isolated after dehydration and hydrolysis.

In view of the difficulties encountered in building up the side-chain with the terminal dehydro-ring already present, attention was directed towards the introduction of the extra double bond with the C_{20} acid (X; vitamin A_1 acid; Arens and van Dorp, *Rec. Trav. chim.*, 1946, **65**, 338) as its methyl ester (now obtained crystalline). The *N*-bromosuccinimide reaction proceeded very readily at 0° or below in chloroform solution. Immediate dehydrobromination of the unstable bromo-compound with 4-phenylmorpholine, followed by chromatography, gave a 75% yield of an ester, hydrolysed to the crystalline dehydro-acid (XI; R = H; vitamin A_2 acid), obtained in 25% overall yield.



With diazomethane this acid gave a crystalline methyl ester (presumably all-*trans*-configuration of the side-chain), the light absorption of which was almost identical with that of the chromatographically purified ester from the dehydrobromination reaction. The latter ester, however, could not be induced to crystallize and is probably a mixture of geometrical isomers—this would also account for the low yield of the pure vitamin A_2 acid produced on hydrolysis. Some experiments, in which bromination had been incomplete, gave a mixture of esters of vitamin A_1 and A_2 acids, which could not be separated chromatographically.

TABLE 1. *Comparison of the light-absorption properties of compounds in the A_1 and A_2 series.*

	A_1 Series		A_2 (Dehydro) Series	
	$\lambda_{max.}$ (Å)	$\varepsilon_{max.}$	$\lambda_{max.}$ (Å)	$\varepsilon_{max.}$
C_{17} Acids	3250	32,500	2865	13,800
			3615	30,000
C_{17} Alcohols	2890	21,500	2690	15,700
			3170	18,100
C_{18} Ketones	3450	28,500	3035	10,300 *
			3840	25,200
,, , semicarbazones	3420	60,000	2975	19,000
			3660	49,500
C_{20} Acids	3500	43,300	3050	12,600 *
			3725	39,300
,, , methyl esters	3590	44,600	3080	13,100 *
			3770	40,600
C_{20} Alcohols	3245	51,000	2880	21,900
			3520	41,300

* Inflexion.

Lithium aluminium hydride reduction of either the crystalline or the non-crystalline methyl ester of the C_{20} dehydro-acid afforded the alcohol (II), the light absorption of which, and of its antimony trichloride colour, agreed closely in both cases with that of natural vitamin A_2 as given by Shantz (*loc. cit.*) (Table 2 and Fig. 1) (for biological activity, see Experimental). This dehydro-alcohol is very much more sensitive to oxygen than is vitamin A_1—a similar relation was observed with other pairs of A_1 and A_2 compounds and may be connected with the possibility of forming transannular peroxides from the dehydro-compounds. The melting point of the *p*-phenylazobenzoate of the alcohol prepared by reduction of the crystalline ester was similar to that recorded by Shantz. However, the melting-point behaviour of the derivative prepared from the non-crystalline (but spectrographically pure) ester suggested that it was a mixture of stereoisomers. It may be noted that the melting point of *neo*- (or *cis*-)vitamin A_1 *p*-phenylazobenzoate (94—96°) is higher than that (79—80°) of the normal (all-*trans*-)vitamin A_1 derivative. Analogous differences

between the vitamin A_2 derivatives would reconcile the apparently conflicting melting-point data of Shantz and Karrer (see Table 2). It seems likely then that both stereoisomeric *p*-phenylazobenzoates were obtained from the non-crystalline ester, thus reinforcing the conclusion reached above. Lithium aluminium hydride reduction of the methyl esters of the C_{17}-acid and the C_{17} dehydro-acid gave the corresponding alcohols, characterized as *p*-phenylazobenzoates.

TABLE 2. *Comparison between natural and synthetic vitamin A_2 and anhydro-vitamin A_2.*

		Natural			Synthetic	
A_2 Series.	M. p.	λ (Å)	ε	M. p.*	λ (Å)	ε
Vitamin	—	3510 (max.)	41,400	—	3520 (max.)	41,300
		2970 (min.)	16,200		2975 (min.)	15,100
		2870 (max.)	23,300		2880 (max.)	21,900
$SbCl_3$ colour in $CHCl_3$	—	6930 (max.)	$E^{1\%}_{1cm.}$ 4100	—	6930 (max.)	$E^{1\%}_{1cm.}$ 4000
p-Phenylazobenzoate	76—77° [1]	3410 (max.)	58,500	78—90° [3]	3410 (max.)	59,500 [3]
	94—95 [2]	—	—	73—77 [4]	—	—
Anhydro-A_2 Series.						
Anhydro-vitamin	89·5 [1]	3350 (infl.)	27,000	87—88	3350 (infl.)	27,500
		3520 (max.)	54,400		3515 (max.)	54,400
		3700 (max.)	79,500		3700 (max.)	81,000
		3910 (max.)	69,700		3915 (max.)	73,000
$SbCl_3$ colour in $CHCl_3$	—	6930 (max.)	$E^{1cm.}_{1\%}$ 4400	—	6930 (max.)	$E^{1cm.}_{1\%}$ 4500

[1] Shantz (*loc. cit.*). [2] Karrer and Schneider (*loc. cit.*). [3] Prepared from non-crystalline dehydro-ester. [4] Prepared from crystalline dehydro-ester.

* Determined on a Kofler block; the *p*-phenylazobenzoate with m. p. 78—90° melted at 84—87° in a capillary tube in the usual paraffin-bath apparatus.

Vitamin A_2, like vitamin A_1, is very sensitive to traces of mineral acids, being converted into an anhydro-compound by loss of the elements of water. Shantz (*loc. cit.*) obtained crystalline anhydro-vitamin A_2, and dehydration of the synthetic material similarly yielded anhydro-vitamin A_2, the physical properties being in excellent agreement with those given by Shantz (Table 2).

Morton (*Nature*, 1944, **153**, 69) suggested that retinene$_1$ and retinene$_2$, which had been isolated from retinal tissue of marine and freshwater fish respectively, might be the aldehydes corresponding to vitamins A_1 and A_2. He later showed that they could be readily prepared by oxidation of the vitamins with a suspension of manganese dioxide in light petroleum (Ball, Goodwin, and Morton, *Biochem. J.*, 1948, **42**, 516; Morton, Salah, and Stubbs, *loc. cit.*). Manganese dioxide oxidation of synthetic vitamin A_2 (prepared *via* the crystalline dehydro-ester) afforded crystalline retinene$_2$, agreeing closely in its properties with those of the aldehyde prepared from the natural vitamin (cf. Table 3). Synthetic retinene$_2$ readily formed an oxime and a 2 : 4-dinitrophenylhydrazone, corresponding derivatives also being prepared from retinene$_1$ for comparison. The oximes, which

TABLE 3. *Melting points and light absorption (in ethanol) of retinene$_1$ and retinene$_2$ and their derivatives.*

	M. p.	$\lambda_{max.}$ (Å)	$\varepsilon_{max.}$	M. p.	$\lambda_{max.}$ (Å)	$\varepsilon_{max.}$
	Retinene$_1$ series.			*Retinene$_2$ series.*		
Retinene	64—65°	3850	40,300	77—78°	3150 * ‡	11,800
					4000	38,000
	(61—62)	(3855)	(39,800)	[77—78]	[3150] *	[—]
					[4000]	[39,500]
Oxime	134—136	3590	57,800	141—143	3080	16,100
					3775	53,400
2 : 4-Dinitrophenylhydrazone	214—215°	4510 †	50,100	197—199	4620 †	46,300
	(207—208)	(4420)	(54,000)			
$SbCl_3$ colour in $CHCl_3$					7300 → 7050	$E^{1\%}_{1cm.}$ 3750
					[7350 → 7050]	[3720]

Data in parentheses are those reported by Ball, Goodwin, and Morton (*Biochem. J.*, 1948, **42**, 516). Data (unpublished) in square brackets were kindly provided by Professor R. A. Morton, the retinene$_2$ having been prepared from natural vitamin A_2.

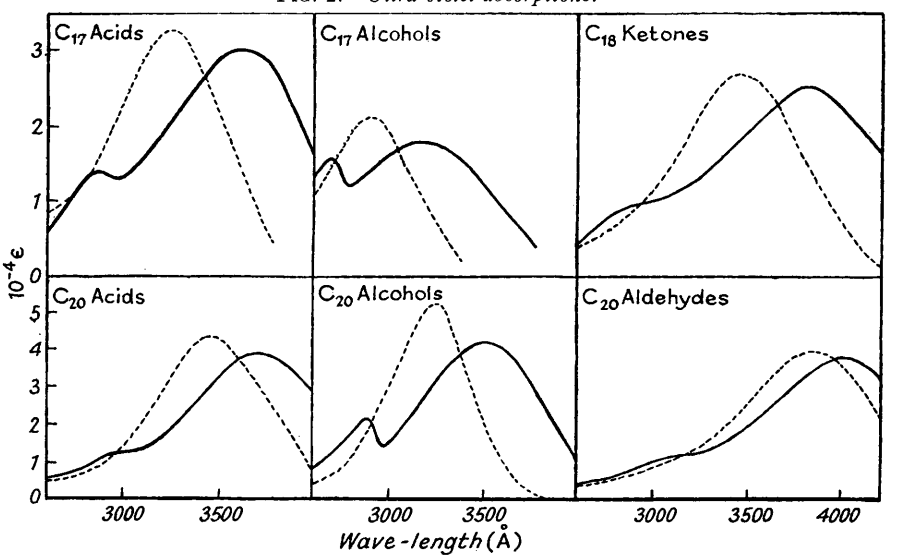
* Inflexion. † Light absorption in chloroform solution.

‡ Further light absorption data for this compound are given in the Experimental section.

crystallized well and were formed in good yields are the most satisfactory type of derivative of these polyene carbonyl compounds. The position of the main absorption maximum of retinene$_2$ 2 : 4-dinitrophenylhydrazone is in accordance with those of polyene aldehyde and ketone derivatives with fewer double bonds, this series obeying the usual $\lambda^2 \propto n$ relation. However, care was necessary in order to prepare a derivative with the correct absorption spectrum, since longer periods of contact with the reagent solution or simple heating of a solution (*e.g.*, in ethyl acetate) of the derivative caused the position of $\lambda_{max.}$ to move to shorter wave-lengths. The analytical composition of the derivative had undergone no change, but the melting point was markedly lower (163—167°). It appears that the material described as retinene$_2$ 2 : 4-dinitrophenylhydrazone by Morton, Salah, and Stubbs (*loc. cit.*) was in fact the foregoing isomerization product—the physical constants are in reasonable agreement (cf. Experimental section).

Oppenauer oxidation of vitamin A$_1$ concentrates with diethyl ketone as the hydrogen acceptor was reported by Howarth, Heilbron, Jones, Morrison, and Polya (*J.*, 1939, 128) to yield a carbonyl compound for which a dehydro-vitamin A$_1$ aldehyde (*i.e.*, now retinene$_2$)

FIG. 2. *Ultra-violet absorptions.*



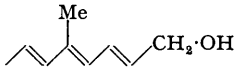
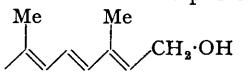
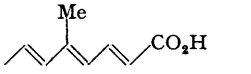
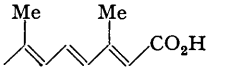
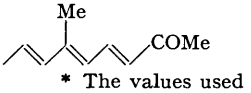
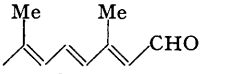
Broken curves : compounds of vitamin A$_1$ series.
Full curves : compounds of vitamin A$_2$ series.

structure was suggested. A similar oxidation of retinene$_1$ has been reported to give the same product (Morton, Salah, and Stubbs, *loc. cit.*). Repetition of these experiments has shown that the compound formed is the (crystalline) C$_{25}$ ketone produced by condensation of the initially formed retinene$_1$ with diethyl ketone—further details of this and related experiments will be reported in a separate communication.

Light Absorption Properties.—Introduction of the extra double bond into the terminal ring invariably moves the position of the main absorption maximum to longer wave-lengths and also decreases the intensity of the main band (see Table 1 and Fig. 2). At the same time a second maximum (sometimes an inflexion) appears in the 2700—3200-Å region, its position moving towards longer wave-lengths as the length of the unsaturated side chain is increased. It is likely that this subsidiary band represents the combined absorption of two " partial chromophores " (together with the tail of the main absorption band); thus in vitamin A$_2$ itself, the partial chromophores (see below for more detailed discussion) are the trimethyl*cyclo*hexadienyl system ($\lambda_{max.} \sim 2700$ Å) and the tetraene side-chain ($\lambda_{max.} \sim 3050$ Å)—equal intensities being assumed for the two chromophores, the combined absorption would give rise to a maximum at ~2870 Å, close to the observed value (it is realized that the side-chain chromophore should contribute a greater share

since its intensity of absorption would be expected to be greater than that of the cyclic chromophore, but more exact computations are not justified at present). Table 4 gives a comparison of the experimentally observed positions of $\lambda_{max.}$ of the subsidiary band with those calculated as an average of two partial chromophores, the trimethyl*cyclo*hexadienyl group being assumed in each case to " contribute " $\lambda_{max.}$ 2700 Å.

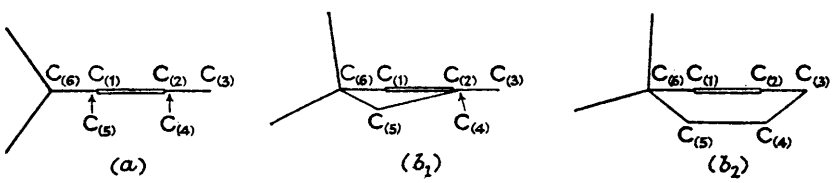
TABLE 4.

Side-chain chromophore	Calc.* average $\lambda_{max.}$ (Å)	Observed $\lambda_{max.}$ (Å)	Side-chain chromophore	Calc.* average $\lambda_{max.}$ (Å)	Observed $\lambda_{max.}$ (Å)
	2675	2690		2875	2880
	2875	2865		3025	3050
	2975	3035		3150	3150

* The values used for the side-chain partial chromophores were either (*a*) those given by Braude (*Ann. Reports*, 1945, **42**, 105) for appropriate acyclic compounds, or (*b*) calculated from such data by addition of 50 or 100 Å per methyl group, depending on the position of the group.

It has been suggested that, in compounds containing a trimethyl*cyclo*hexenyl ring, the effectiveness of the conjugation between the cyclic double bond and the side chain is reduced by steric factors (Braude, Jones, Koch, Richardson, Sondheimer, and Toogood, *J.*, 1949, 1890). The chief result with the compounds examined was a reduction in absorption intensities, but some evidence was obtained of absorption due to partial chromophores produced by a break in conjugation between the ring olefinic bond and the side-chain (*e.g.*, the absorption of β-ionone and dehydro-β-ionone in the 2200-Å region due to the side-chain C=C–C=O absorption). In more extended polyene systems the side-chain partial chromophore probably lies too close to the main band to be resolved. In this connection, Oroshnik, Karmas, and Mebane (*J. Amer. Chem. Soc.*, 1952, **74**, 295) have pointed out that the absorption bands of vitamin A_1 and its congeners are hypsochromically displaced relatively to acyclic polyenes, the observed absorption bands being in fact a combination of the complete chromophore (of reduced intensity) and the partial chromophore containing one less double bond.

FIG. 3.



On the assumption that the side-chain is attached to the ring in the energetically more favourable *s-trans*-configuration (see also MacGillavray, Kreuger, and Eichhorn, *Kon. Ned. Akad. Wet.*, 1951, *B*, **54**, 449), the break in conjugation in the A_1 series of compounds is clearly caused by interference between the *gem*-dimethyl group and the side-chain $C_{(8)}$-hydrogen atom. In the dehydro-series, the decrease in intensity of the main absorption band (relatively to the A_1 series), as well as the appearance of well-defined partial chromophoric bands, indicates an even greater steric inhibition of resonance.

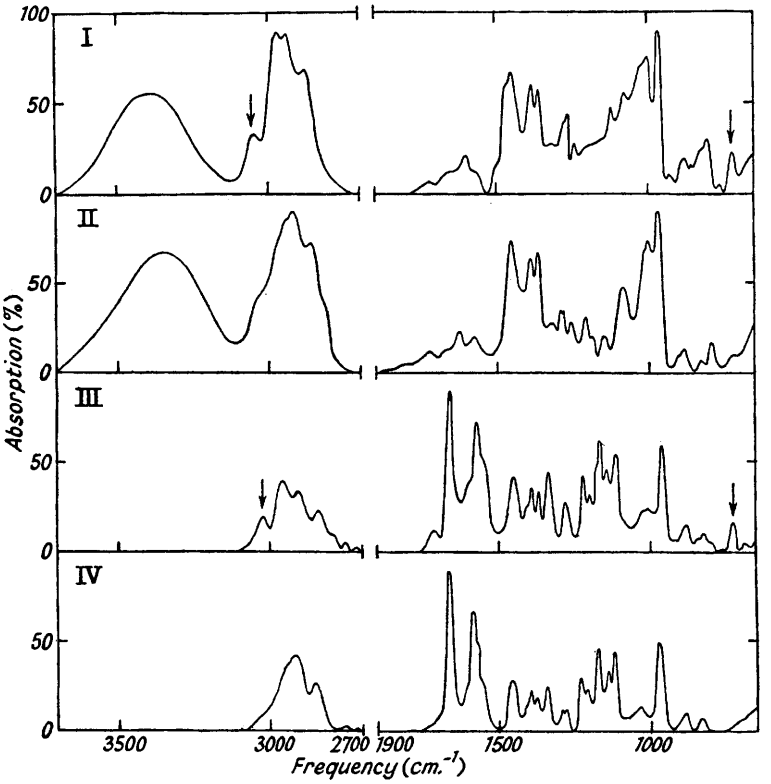
This, it is believed, is connected with the greater rigidity of *cyclo*hexadiene rings compared with *cyclo*hexene rings. With the planar *cyclo*hexadiene ring, models confirm that appreciable " overlap " takes place between the $C_{(8)}$-hydrogen and the *gem*-dimethyl group, the latter (regarded effectively as a single atomic grouping) being in exactly the same plane as the ring (Fig. 3*a*), and hence the side chain. In the A_1 series, the more flexible *cyclo*hexene ring can assume a skew configuration (Fig. 3b_1) or a form (b_2) similar

to the boat form of *cyclo*hexane, but in either case, the *gem*-dimethyl group is located above (or below, ready interconversion being probable) the general plane of the ring. Interference of the *gem*-dimethyl group and the $C_{(8)}$-hydrogen atom will thus be reduced.

Infra-red Spectra.—The infra-red spectra of the compounds prepared during this investigation are presented in Table 5 (see also Fig. 4). Dr. Shantz kindly sent us (unpublished) infra-red spectra of natural vitamin A_2 and anhydro-vitamin A_2. Close agreement was observed between the spectra of the natural and synthetic compounds.

Comparison of the A_2 with the A_1 series shows that the cyclic *cis*-double bond present in the former gives rise to absorption peaks at ~725 cm.$^{-1}$ and ~3030 cm.$^{-1}$. Some of the A_1

FIG. 4. *Infra-red absorption curves.*



I, *Vitamin* A_2. III, *Retinene*$_2$.
II, *Vitamin* A_1. IV, *Retinene*$_1$.

compounds also showed a peak near 725 cm.$^{-1}$, but invariably of lower intensity; vitamins A_1 and A_2 themselves showed the smallest difference in this region. The distinct peak near 3030 cm.$^{-1}$ (*cis*-double bond C–H stretching frequency) shown by all the dehydro-compounds could be easily distinguished from a shoulder present in this region of the spectra of the A_1 compounds due to the C–H stretching frequency of the *trans*-disubstituted double bonds.

In the compounds containing a carbonyl group in conjugation with the polyene chain, the olefinic bond stretching frequency decreases (from 1605 cm.$^{-1}$ in β-ionone to 1572 cm.$^{-1}$ in retinene$_2$) as the number of double bonds increases. However, the relation between the carbonyl stretching frequency (1600—1700-cm.$^{-1}$ region) and the number of double bonds shows much less regularity. These results are thus parallel to those obtained with simpler unsaturated carbonyl compounds (Blout, Fields, and Karplus, *J. Amer. Chem. Soc.*, 1948, **70**, 194).

TABLE 5. *Infra-red frequencies (cm.$^{-1}$) for compounds of the vitamin A_1 and A_2 series.*

	3700—3100	3100—3000 *	3000—2900	2900—2800	2800—1700	1700—1500	1500—1400
1. β-Ionone	—	—	2940 s	2873 ms	—	1685 ms 1666 s	1605 ms 1460 mw
2. Dehydro-β-ionone	—	3065 m	2983 s 2950 s	2885 ms 2827 m	—	1665 s	1598 s 1465 m
3. C_{17}-Alcohol	3400 ms	—	2940 s	2870 ms	—	1665 m	1455 ms
4. C_{17} Dehydro-alcohol	3380 ms	3035 m	2960 s 2930 s	2865 ms	—	1660 m	1460 ms 1445 ms
5. Ester of C_{17} acid	—	—	2940 ms	2860 m	1710 s	1620 ms	1600 ms 1435 ms
6. Ester of C_{17} dehydro-acid	—	3030 m	2945 ms	2855 m 2805 w	1703 s	1615 ms	1592 ms 1435 ms
7. C_{18} Ketone	—	—	2940 ms	2870 m	—	1680 m 1660 m	1588 ms 1445 mw 1568 ms
8. C_{18} Dehydro-ketone	—	3030 mw	2955 ms 2918 ms	2875 m 2802 w	—	1675 ms 1655 ms	1585 ms 1442 mw 1562 ms
9. Vitamin A_1	3350 ms	—	2970 s	2860 ms	—	1620 w	1572 w 1452 ms
10. Vitamin A_2	3400 ms (3400)	3050 m	2975 s 2940 s (2960)	2880 ms	—	1600 w (1600)	1450 ms (1450)
11. Retinene$_1$	—	—	2910 ms	2845 m —	2705 w 2747 w	1655 s	1575 ms 1445 m
12. Retinene$_2$	—	3020 mw	2940 m 2910 m	2840 m —	2710 w 2750 w	1655 s	1572 ms 1447 m
13. Ester of C_{20} acid	—	—	2930 ms	2860 m	1708 s	1608 m	1582 ms 1440 m
14. Ester of C_{20} dehydro-acid	—	3053 mw	2960 ms	2880 m	1711 s	1610 ms	1585 ms 1438 m
15. Anhydro-vitamin A_1	—	—	2930 s 2910 s	—	1780 w	1610 m	1560 mw 1450 ms
16. Anhydro-vitamin A_2	—	—	2980 s 2940 s	2870 ms	1780 w	1612 mw	1450 m (1450)

	1400—1300	1300—1200	1200—1100	1100—1000	1000—900	900—750	750—650
1. β-Ionone	1360 ms	1257 s	1177 w	—	980 ms	—	—
2. Dehydro-β-ionone	1361 s	1253 s	1170 m	—	980 ms	—	721 m
3. C_{17} Alcohol	1383 m 1361 ms	—	—	1090 m 1042 ms	968 s	—	—
4. C_{17} Dehydro-alcohol	1380 ms 1360 ms	—	—	1087 m 1042 ms	962 s	—	723 m
5. Ester of C_{17} acid	1360 m 1315 ms	1270 s 1222 m	1148 s 1132 ms	1040 mw	975 ms	890 w 855 w	720 w
6. Ester of C_{17} dehydro-acid	1360 m 1312 ms	1276 s 1221 m	1146 s 1130 s	1040 mw	976 ms	890 w 855 w	730 w 718 mw
7. C_{18} Ketone	1360 m	1275 ms 1250 s	1150 mw	—	970 ms	890 w	720 w
8. C_{18} Dehydro-ketone	1360 ms	1275 ms 1250 s	1150 mw	—	970 ms	890 w	725 mw 678 w
9. Vitamin A_1	1380 ms 1358 ms	1280 mw 1270 mw	—	1080 m 1000 ms	965 s	880 w 788 w	720 w
10. Vitamin A_2	1380 ms 1360 m (1390)	1260 mw (1240)	—	1070 m 1000 ms (1080) (1020)	965 s (965)	880 w 800 mw (790)	720 w
11. Retinene$_1$	1381 mw 1330 m 1360 mw	1215 m	1160 ms 1112 ms	—	964 ms	880 w 820 w	—
12. Retinene$_2$	1385 mw 1332 m 1357 mw	1213 m	1159 ms 1111 ms	—	962 ms	875 w	726 w
13. Ester of C_{20} acid	1354 m	1240 ms	1150 s	—	965 ms	—	—
14. Ester of C_{20} dehydro-acid	1355 m	1240 ms	1150 s	—	962 ms	—	720 w
15. Anhydro-vitamin A_1	1390 m 1360 ms	1200 m	—	1080 mw	982 ms 962 s	882 s 827 mw	665 w
16. Anhydro-vitamin A_2	1390 mw 1310 mw 1362 m (1390) (1310) (1360) (1300)	1200 ms (1180)	—	1085 s 1102 s (1090)	985 ms 968 s (990) (960)	885 ms 830 w (885) (830)	—

(Footnotes to Table on opposite page.)

A discussion of the structures and infra-red spectra of anhydrovitamins A_1 and A_2 is deferred.

Meunier (*Rev. internat. Vitaminologie*, 1951, **23**, 21) has claimed on the basis of qualitative ultra-violet absorption spectra and growth-promotion tests that manganese dioxide oxidation of lycopene gives rise to retinene$_2$, this being suggested as evidence in favour of the open-chain structure for vitamin A_2. The absorption curve given (3000—4600 Å) for the suggested retinene$_2$ does not exhibit the characteristic inflexion at 3550 Å, and further comment on Meunier's experiments must be deferred until the properties of pure reaction products are fully described.

Experimental

M. p.s were determined on a Kofler block and are corrected. Ultra-violet light absorption measurements were made in ethanol with a Beckman spectrophotometer unless stated otherwise; infra-red determinations were made with a Perkin–Elmer double-beam instrument, model 21 (sodium chloride prism), the material being used as either a liquid or a supercooled melted solid.

Chromatographic alumina (P. Spence, Grade H) was deactivated by addition of a certain amount of water, or neutralized and deactivated by addition of dilute acetic acid (10% in water)—the material then being shaken until homogeneous. The resultant free-flowing alumina was used as a suspension in light petroleum to fill columns. This method of " neutralizing " alumina proved to be very efficacious for chromatography of the methyl esters handled in this work (and also for esters generally), hydrolysis being prevented during periods up to 24 hours. Chromatography of the most sensitive compounds, such as vitamin A_2 and retinene$_2$, was conducted in a cold room at $-8°$ to $-10°$ in subdued light.

Solvents were always evaporated under reduced pressure. Light petroleum refers to the fraction with b. p. 40—60°. Chloroform used in the bromination reaction was freshly dried by distillation from phosphoric oxide. *N*-Bromosuccinimide was powdered to pass through a 125-mesh sieve. Most experiments were carried out in a nitrogen atmosphere.

Condensation of Dehydro-β-ionone with Methyl γ-Bromocrotonate.—A mixture of dehydro-β-ionone (0·57 g.), methyl γ-bromocrotonate (0·9 g.), zinc dust (0·5 g.), and dry benzene (10 c.c.) was heated under reflux. After 10 minutes, the reaction commenced; heating was then continued for a further 30 minutes. Treatment with dilute acetic acid and ice gave the crude hydroxy-ester (0·7 g.), $\lambda_{max.}$ 2890 Å ($\varepsilon = 6000$) (cf. light absorption of dehydro-β-ionol, $\lambda_{max.}$ 2850 Å; $\varepsilon = 8400$; Henbest, *loc. cit.*). A solution of this ester in benzene (10 c.c.) containing toluene-*p*-sulphonic acid (3 mg.) was heated under reflux for 30 minutes. The resultant gum had $\lambda_{max.}$ 3350 Å ($E^{1\%}_{1cm.} = 350$). Alkaline hydrolysis afforded no crystalline acid.

5-Methyl-7-(2 : 6 : 6-trimethylcyclohexa-1 : 3-dienyl)hepta-2 : 4 : 6-trienoic Acid (C_{17} *Dehydro-acid*) (VII; R = H).—A solution of diazomethane in ether was added to a suspension of the C_{17} acid (5·0 g.) (prepared by the procedure given by Inhoffen, Bohlmann, and Bartram, *Annalen*, 1948, **561**, 13) in ether at 20° until no more nitrogen was evolved. The solvent was removed, and the residue dissolved in pentane, filtration and evaporation giving the methyl ester (5·12 g.). This was dissolved in chloroform (50 c.c.) at 15° and powdered *N*-bromosuccinimide (3·86 g.) was then added with vigorous stirring. After 20 minutes the bromo-imide had dissolved, 4-phenylmorpholine (4·0 g.) was added, and the solution was heated under reflux for 10 minutes. Dilute hydrochloric acid and pentane were added to the cooled solution. The pentane–chloroform layer was evaporated and the residue, dissolved in pentane (10 c.c.), was chromatographed on deactivated alumina (2000 g.). The column was developed with light petroleum until separation of the bands appeared complete, the main yellow band being then cut out and eluted with ether, to give the crude dehydro-ester (4·8 g.) as a deep yellow oil. This was hydrolysed under reflux in methanolic potassium hydroxide (25 c.c.; 10% w/v) for 45 minutes. After addition of water, the non-saponifiable material was removed by pentane-extraction, and the aqueous layer acidified with dilute hydrochloric acid and extracted with pentane–ether (1 : 1). Evaporation gave crude acid (4·45 g.), which after recrystallization

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s = Strong, m = medium, and w = weak band—these are given only as approximate indications of absorption intensity.

Values in parentheses are for natural materials; the curves from 4000 to 770 cm.$^{-1}$ for vitamin A_2, and from 2000 to 770 cm.$^{-1}$ for anhydro-vitamin A_2 were kindly supplied by Dr. E. M. Shantz.

* All the A_1-compounds (except β-ionone) showed a definite shoulder at about 3040 cm.$^{-1}$.

from acetone gave the C_{17} *dehydro-acid* (2·8 g., 55%) as golden-yellow needles (prisms), m. p. 163—164° (Found: C, 78·75; H, 8·7. $C_{17}H_{22}O_2$ requires C, 79·05; H, 8·6%).

5-Methyl-7-(2 : 6 : 6-trimethylcyclohex-1-enyl)hepta-2 : 4 : 6-trien-1-ol (C_{17} *Alcohol*).—A solution of the ester, prepared as above from C_{17} acid (1 g.), in ether (1 c.c.) was treated at −30° with an ethereal solution of lithium aluminium hydride (4·5 c.c.; 0·55M) for 2 hours. The reaction mixture was then allowed to warm to 20°, a few drops of ethyl acetate were added, and the whole was poured into saturated aqueous tartaric acid. The product was extracted with pentane–ether (1 : 1), this extract being washed with sodium hydrogen carbonate solution and dried. Removal of the solvent gave an oil which was chromatographed on alumina (100 g.) (deactivated with water, 10%). Material eluted with pentane was discarded; further elution with pentane–ether (1 : 1) gave the alcohol (0·67 g., 75%). A portion was distilled at 95—100° (bath-temp., short-path still)/10^{-4} mm., to yield the pure *alcohol*, n_D^{25} 1·5650 (Found: C, 82·7; H, 10·5. $C_{17}H_{26}O$ requires C, 82·85; H, 10·65%).

p-Phenylazobenzoyl chloride (0·5 g.) was added to a solution of the foregoing alcohol (0·45 g.) in benzene (5 c.c.) containing a few drops of pyridine, the mixture being kept at 20° overnight. *p*-Phenylazobenzoic anhydride was removed by filtration and the benzene solution washed with dilute acid, and sodium hydrogen carbonate solution, dried, and evaporated. The residue was chromatographed on deactivated alumina (100 g.; water, 10%), the ester being eluted with light petroleum. Evaporation afforded a red oil which crystallized from a 20% solution in pentane at −20°, to give the p-*phenylazobenzoate* as orange-red needles (0·25 g.), m. p. 37—38° (Found: C, 79·0; H, 7·5. $C_{30}H_{34}O_2N_2$ requires C, 79·25; H, 7·55%). Light absorption: Maximum, 3200 Å (ε = 36,700).

5-Methyl-7-(2 : 6 : 6-trimethylcyclohexa-1 : 3-dienyl)hepta-2 : 4 : 6-trien-1-ol (C_{17} *Dehydro-alcohol*).—C_{17} Dehydro-acid (0·7 g.) was converted by diazomethane into its methyl ester. This was dissolved in ether (1 c.c.), and treated with lithium aluminium hydride in ether (3·25 c.c.; 0·55M) at −30° for 2 hours. After warming to room temperature, the mixture was processed as described above for the C_{17} alcohol. Chromatographic purification gave a yellow oil (0·31 g., 50%), part of which was distilled at 100—105° (bath-temp., short-path still)/10^{-4} mm., to afford the C_{17} *dehydro-alcohol*, n_D^{21} 1·5824 (Found: C, 83·3; H, 9·7. $C_{17}H_{24}O$ requires C, 83·55; H, 9·9%).

This alcohol (0·15 g.) was treated with *p*-phenylazobenzoyl chloride (0·2 g.) in benzene (5 c.c.) containing a few drops of pyridine as described above. The *p*-phenylazobenzoate crystallized from pentane at −20° as an orange solid, m. p. *ca.* 10—15°. Light absorption: Maximum, 3240 Å (ε = 33,100); inflexion, 2730 Å (ε = 18,700).

6-Methyl-8-(2 : 6 : 6-trimethylcyclohexa-1 : 3-dienyl)octa-3 : 5 : 7-trien-2-one (C_{18} *Dehydro-ketone*) (IX).—A solution of the C_{17} dehydro-acid (1·4 g.) was treated with stirring with an ethereal solution of methyl-lithium (200 c.c.; 0·13N) at 20°. Ice and water were added after ½ hour, the ethereal solution being processed as usual. Evaporation afforded a reddish-orange oil, which was purified by chromatography on alumina (200 g.) (deactivated with water, 3%). Elution with light petroleum gave the *dehydro-ketone* (0·6 g.), prepared for analysis by removal of solvent at 10^{-5} mm. at 20° (Found: C, 83·95; H, 9·6. $C_{18}H_{24}O$ requires C, 84·35; H, 9·45%). The *semicarbazone* crystallized from methanol as deep yellow rhombs, m. p. 187—190° (Found: C, 72·95; H, 8·45; N, 13·3. $C_{19}H_{27}ON_3$ requires C, 72·8; H, 8·7; N, 13·4%). The 2 : 4-*dinitrophenylhydrazone* crystallized from ethyl acetate as dark red laths, m. p. 168—170° (Found: N, 12·7. $C_{24}H_{28}O_4N_4$ requires N, 12·85%). Light absorption (in chloroform): Maximum, 4330 Å (ε = 41,700); inflexion, 3700 Å (ε = 21,800).

Methyl 3 : 7-*Dimethyl-9-(2 : 6 : 6-Trimethylcyclohex-1-enyl)-2 : 4 : 6 : 8-tetraenoate* (*Methyl Ester of Vitamin* A_1 *Acid*) (X; R = Me).—A solution of diazomethane in ether was added to a suspension of vitamin A_1 acid (5·4 g.) in ether until no more nitrogen was evolved. Removal of the solvent gave a residue that readily solidified. Recrystallization from methanol–water (6 : 1) with cooling to −8° gave the methyl ester as yellow needles, m. p. 55—56° (Found: C, 80·3; H, 9·75. $C_{21}H_{30}O_2$ requires C, 80·2; H, 9·6%).

3 : 7-*Dimethyl-9-(2 : 6 : 6-trimethylcyclohexa-1 : 3-dienyl)nona-2 : 4 : 6 : 8-tetraenoic Acid* (C_{20} *Dehydro-acid*) (XI; R = H).—Powdered *N*-bromosuccinimide (1·3 g.) was added all at once to a vigorously stirred solution of the methyl ester (2 g.) of vitamin A_1 acid in dry chloroform (20 c.c.) at 0°. After about 7 minutes a brown colour developed but stirring was continued for a total of 15 minutes, the mixture being kept at 0°. 4-Phenylmorpholine (2 g.) was added and the solution heated under reflux for 5 minutes, whereafter it was poured into dilute hydrochloric acid and pentane. The pentane extract was treated in the usual way, and the product chromatographed on deactivated alumina (400 g.). The column was developed with light

petroleum and, when the main yellow band had separated, the subsidiary coloured bands above it were removed with a spatula. The dehydro-ester could then be eluted rapidly with ether–light petroleum (1 : 1), evaporation giving an orange oil (1·48 g.) with light absorption : Maximum, 3770 Å (ε = 38,600). This ester was hydrolysed by heating it under reflux in methanolic potassium hydroxide (30 c.c. of 10%) for 1 hour. The reaction mixture was poured into water and, after neutral material had been removed by extraction with pentane, the solution was acidified with dilute hydrochloric acid, and the acid product isolated with ether. The residue (1·38 g.) on crystallization from acetone gave the C_{20} *dehydro-acid* (0·46 g.) as orange-yellow needles, m. p. 175—177° (Found : C, 80·15; H, 9·0. $C_{20}H_{26}O_2$ requires C, 80·4; H, 8·8%). Ethereal diazomethane gave the *methyl* ester, which crystallized as fine yellow needles from a small volume of methanol cooled to −20°. After two recrystallizations the m. p. was 45—47° (Found : C, 80·3; H, 9·1. $C_{21}H_{28}O_2$ requires C, 80·7; H, 9·05%).

3 : 7-*Dimethyl*-9-(2 : 6 : 6-*trimethyl*cyclo*hexa*-1 : 3-*dienyl*)*nona*-2 : 4 : 6 : 8-*tetraen*-1-*ol* (*Vitamin* A_2) (II).—Crystalline methyl ester (0·224 g.) of the C_{20} dehydro-acid was dissolved in dry ether (1 c.c.), the solution then being cooled to −30° in a flask fitted with a guard-tube containing potassium hydroxide pellets. Lithium aluminium hydride in ether (1·2 c.c.; 0·45M) was then added, and the reaction mixture kept at −30° for 3 hours. It was then allowed to warm to 20°, 2 drops of ethyl acetate were added, and the mixture was treated with aqueous tartaric acid and ether–pentane (1 : 1). The product was chromatographed on alumina (100 g.) (deactivated with water, 10%). Elution with pentane removed unchanged ester, together with traces of aldehyde (retinene$_2$), the vitamin being eluted finally with ether–pentane (1 : 1). Evaporation gave vitamin A_2 (0·098 g.) as a yellow oil. Light absorption, see Table.

α-Tocopherol could be added to the vitamin during its isolation and purification in order to diminish its extreme sensitivity towards traces of oxygen, which rapidly reacts with the vitamin to form a white amorphous material insoluble in light petroleum.

The reaction between the above vitamin (52 mg.) and *p*-phenylazobenzoyl chloride (60 mg.) in benzene (2 c.c.) containing a little pyridine was carried out as described for the C_{17}-alcohol derivative. After 3 recrystallizations from pentane at −20°, the *p*-phenylazobenzoate was obtained as orange needles, m. p. 73—77°.

A similar reduction of non-crystalline methyl ester (0·225 g.) [purified by chromatography after dehydrobromination of the intermediate 3-bromo-compound (see above)] with lithium aluminium hydride in ether (3·8 c.c. of 0·45M) gave, after the same isolation procedure, vitamin A_2 (0·10 g.), with the same light absorption properties. Formation of the *p*-phenylazobenzoate gave a product with m. p. 78—90° [m. p. (capillary) 84—87°] after 3 recrystallizations from light petroleum.

A solution of the (all-*trans*-)vitamin A_2 in arachis oil showed 30% of the activity of vitamin A_1 when tested on vitamin-A deficient rats—for this test we are indebted to Dr. W. F. J. Cuthbertson of Glaxo Laboratories Ltd. Shantz and Brinkmann (*J. Biol. Chem.*, 1950, **183**, 467) reported that natural vitamin A_2 has 40% of the activity of vitamin A_1.

Anhydrovitamin A_2.—Vitamin A_2 (0·36 g.) obtained as described above from non-crystalline methyl ester was dissolved in dry methanol (5 c.c.), and dry methanolic hydrogen chloride (40 c.c.; M/30) was added. The solution was kept at 20° for 20 minutes, then water and pentane were added. The crude anhydro-compound was chromatographed on alumina (100 g.) (deactivated with water, 10%), the column being eluted with pentane. A single yellow band moved rapidly down the column; elution and evaporation afforded a yellow oil (0·25 g.). This was dissolved in pentane (1 c.c.) and filtered through a small column of alumina (0·2 g.). The solution was cooled to −30° and kept at this temperature for 5 hours, and then at −10° overnight whereupon a solid separated. The supernatant liquid was removed at −10° with a dropper and the product recrystallized twice in the same manner, to give the hydrocarbon as small yellow prisms (75 mg.), m. p. 87—88°. Vitamin A_1 (0·85 g.), when treated in the same way, gave anhydrovitamin A_1 (0·16 g.) as pale yellow prisms, m. p. 77°.

3 : 7-*Dimethyl*-9-(2 : 6 : 6-*trimethyl*cyclo*hexa*-1 : 3-*dienyl*)*nona*-2 : 4 : 6 : 8-*tetraenal* (*Retinene*$_2$). —A solution of vitamin A_2 (0·214 g.; prepared from crystalline methyl ester of the C_{20} dehydro-acid) in pentane (200 c.c.) was shaken with manganese dioxide (5 g.) in a stoppered bottle for 18 hours. The product obtained by filtration and evaporation was chromatographed on alumina (100 g.) (deactivated with water, 10%). Elution with pentane–ether (100 : 1) caused the orange retinene$_2$ band to move slowly down the column. Elution with this solvent mixture, followed by evaporation of the solvent, gave an orange-red oil (0·176 g.), which was dissolved in pentane (0·9 c.c.) and filtered through a short pad of alumina (0·2 g.) into a small tube. The solution was cooled to −30° and then allowed to stand at −10° overnight. The mother-liquor

was removed from the solid retinene$_2$ by means of a dropper, and the compound was twice recrystallized from warm (30°) pentane by cooling to 0°. Retinene$_2$ (93 mg.) was obtained as orange-red prisms, m. p. 77—78° (Found : C, 84·75; H, 9·0. $C_{20}H_{26}O$ requires C, 85·05; H, 9·3%). Light absorption in pentane : Maximum, 3880 Å ($\varepsilon = 41{,}100$). In chloroform : Maximum, 4070 Å ($\varepsilon = 36{,}500$). Morton (private communication) gives respectively for these solvents : Maximum, 3860 ($\varepsilon = 40{,}900$) and 4080 Å ($\varepsilon = 38{,}200$).

A solution of retinene$_2$ (41 mg.) in warm methanol (2 c.c.) was treated with hydroxylamine acetate [from hydroxylamine hydrochloride (0·5 g.) in water (0·5 c.c.) and potassium acetate (1 g.) in methanol (2·5 c.c.)], and the mixture, after gentle warming, was kept at 0° for 4 hours. The solvent was removed under reduced pressure, and the residue washed with a little cold pentane and then water. The product, recrystallized from methanol, yielded *retinene$_2$ oxime* (28 mg.) as dark yellow needles, m. p. 141—143° (Found : C, 80·95; H, 9·3. $C_{20}H_{27}ON$ requires C, 80·75; H, 9·15%).

A solution of retinene$_2$ (22 mg.) in methanol (1 c.c.) was treated with a few drops of 2 : 4-dinitrophenylhydrazine sulphate reagent [2 : 4-dinitrophenylhydrazine (2 g.) in methanol (100 c.c.) containing sulphuric acid (7 c.c.)]. Water and benzene were quickly added, and the organic layer washed with water, dried (Na_2SO_4), and evaporated. The product was chromatographed on alumina (100 g.) (deactivated with water, 10%), and the deep red band eluted with benzene. After removal of solvent the residue was recrystallized by dissolving it in ethyl acetate at 20° and partly evaporating (and hence cooling) the solvent under reduced pressure. *Retinene$_2$ 2 : 4-dinitrophenylhydrazone* was obtained as violet-black needles, m. p. 197—199° (Found : C, 67·3; H, 6·7. $C_{26}H_{30}O_4N_4$ requires C, 67·55; H, 6·55%).

A solution of retinene$_2$ 2 : 4-dinitrophenylhydrazone (1·001 mg.) in chloroform (50 c.c.) had $E^{1\%}_{1cm.} = 1000$ at 4620 Å. After being heated under reflux for 30 minutes, the solution showed maximal absorption at 4470 Å with $E^{1\%}_{1cm.} = 866$. From a similar experiment employing ethyl acetate as solvent, a crystalline *substance* was isolated with m. p. 163—167° (Found : C, 67·35; H, 6·8. $C_{26}H_{30}O_4N_4$ requires C, 67·55; H, 6·55%). The absorption maximum of this material in ethanol solution was at 4480 Å. Morton, Salah, and Stubbs (*loc. cit.*) give m. p. 160—161°, and Professor Morton (private communication) has informed us that this product, which was believed to be the "normal" derivative of retinene$_2$, shows maximal light absorption at 4450 Å in ethanol solution.

Retinene$_1$ Oxime and 2 : 4-Dinitrophenylhydrazone.—Crystalline retinene$_1$ (0·3 g.) was prepared from vitamin A_1 (0·825 g.) and manganese dioxide (20 g.) in pentane (250 c.c.) as described above for retinene$_2$. The aldehyde was obtained as orange prisms, m. p. 64—65°. *Retinene$_1$ oxime* was prepared in the same way as retinene$_2$ oxime; it crystallized from methanol as short orange-yellow needles, m. p. 134—136° (Found : C, 80·1; H, 9·8. $C_{20}H_{29}ON$ requires C, 80·25; H, 9·75%). The 2 : 4-dinitrophenylhydrazone separated from ethyl acetate as deep purple needles, m. p. 215°.

The authors are greatly indebted to Dr. H. M. Wuest, Warner Institute for Therapeutic Research, New York, for a generous gift of vitamin A_1 acid, and to Dr. O. Isler, Hofmann–La Roche and Co., Basle, for kindly providing vitamin A_1 acetate. They thank Professor R. A. Morton, F.R.S., and Dr. E. M. Shantz for providing unpublished information, and Dr. M. St. C. Flett of Imperial Chemical Industries Limited, Dyestuffs Division, for some preliminary infra-red measurements on anhydro-vitamin A_2. Dr. G. D. Meakins, of this Department, determined the remainder of the infra-red spectra on the Perkins–Elmer instrument made available through the generosity of the Rockefeller Foundation. One of the authors (J. C. H.) is indebted to the Department of Scientific and Industrial Research for a Maintenance Grant.

THE UNIVERSITY, MANCHESTER, 13. [*Received, March 20th*, 1952.]