and A_2 agree so closely with those of anhydrovitamins A_1 and A_2 as to make it clear that dehydration must here precede formation of the blue 'ionized' form. The 664 mμ. and 735 (705 mμ.) bands of the retinenes belong to ionized forms which can only be formulated speculatively; in any case there is no electrophysiological or chemical evidence to connect them unequivocally with known aspects of visual chemistry. The 'strong acid' peaks in the vitamin A series are plausibly analogous to Granit's photopic modulators. The results on retinene$_2$ neither add to, nor detract from, such plausibility. If, however, the existence of labile ions is held to show that vitamin A_1 is sufficiently versatile to be responsible for photopic vision, a rather important hint is provided by retinene$_2$. The absorption peaks of retinene$_2$ in sulphuric acid occur at nearly the same wavelengths as those of retinene$_1$. If the positions of the photopic modulators were found in the eyes of freshwater fish (or other 'porphyropsin' eyes) to be shifted as compared with those of mammalian or sea-fish eyes, the idea that these coloured ions possess some fundamental characteristics in common with the modulators would lose in force. The agreement between the wavelengths of absorption peaks of the two retinenes in concentrated sulphuric acid is unexpected. Unfortunately, it seems doubtful whether the electrophysiological methods can be made selective enough to provide a firm basis for fine distinctions in the positions of modulators.

To counterbalance the above it must be said that the first requirement in postulating a common key substance for scotopic and photopic vision (i.e. one in which vitamin A_1 or A_2 is equally important to both types of sensitivity) is that it should reproduce the Purkinje shift (500–565 mμ. in vitamin A_1 eyes). The necessary possibilities are provided by the grouping ACH=NR in which ACHO = retinene$_1$ or retinene$_2$, RNH_2 = amino group linked with aliphatic or aromatic residues. Chromophorically A and R could be reversed. Further investigation is, of course, necessary.

SUMMARY

1. Retinene$_2$ with aliphatic amines forms spectroscopic analogues of acid and alkaline indicator yellow$_2$. With aromatic amines it forms compounds which after acidification exhibit a new broad band with $\lambda_{max.}$ 560 mμ.

2. Retinene$_2$ reacts with concentrated sulphuric acid to give coloured unstable products with absorption peaks near 570, 525 and 470 mμ.

3. The bearing of these results on the chemistry of vision is discussed briefly.

We are indebted to the Medical Research Council for financial assistance.

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Studies in Vitamin A

21. RETINENE$_2$ AND VITAMIN A_2

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A preceding paper (Cama *et al.* 1952) records the preparation of crystalline retinene$_2$ from various sources. When retinene$_1$ is fed to rats it is reduced in the lining of the intestine to vitamin A_1 and the esterified vitamin is then carried to the liver and stored (Glover, Goodwin & Morton, 1948). Retinene$_1$ may be reduced to vitamin A_1 either by the Ponndorf method or by the use of lithium aluminium hydride ($LiAlH_4$).

The present paper records the similar conversion *in vivo* and *in vitro* of retinene$_2$ to vitamin A_2. The properties of vitamin A_2 are discussed with particular reference to the rather vexed question of chemical constitution.

EXPERIMENTAL

Feeding experiments with retinene$_2$

Formation of vitamin A$_2$. Groups of young rats (weaned and accustomed to a normal diet) were transferred to a vitamin A- and carotenoid-free diet known from previous experience to result in vitamin A deficiency. When a group began to lose weight, one rat was killed and the liver examined for vitamin A. In all cases the liver reserve was found to be very low and it was usually negligible.

Rats from such groups, when losing weight, were given a dose of retinene$_2$ in arachis oil (0·5–1 ml.); 24 hr. later they were anaesthetized and killed. The livers were then examined for vitamin A$_2$.

Example. Retinene$_2$, m.p. 61° (Cama *et al.* 1952), was dissolved in arachis oil (8 mg./ml.) and a dose of 0·5 ml. given to each rat. The liver from an animal killed 24 hr. later was ground with silver sand and Na_2SO_4 and extracted with redistilled diethyl ether. The ether was removed in a current of N_2 and part of the residue was dissolved in $CHCl_3$. When tested by means of the $SbCl_3$ reagent a greenish blue colour was obtained ($\lambda_{max.}$ 693 mμ. well defined; no 620 mμ. band to be seen; $E_{693\ m\mu.}/E_{620\ m\mu.} = 2{\cdot}7$). When a solution of the liver lipid in *cyclo*hexane was examined for ultraviolet absorption, maxima at 347·5, 287 and 275 mμ. were recorded. With similar preparations chromatographic separations on bone meal showed that the liver lipids contained both esterified and free vitamin A$_2$.

When retinene$_2$ of m.p. 77–78° (Cama *et al.* 1952) was used the results were similar (except that the minor absorption peak at 275 mμ. was not always seen). Vitamin A$_2$ recovered from the livers 18–24 hr. after dosage with retinene$_2$ amounted to about 25% of that theoretically possible. With retinene$_1$ 'yields' of vitamin A$_1$ were nearer 50%.

Using an uncrystallized concentrate of retinene$_2$ (free from retinene$_1$ but by spectrophotometric assay containing 75% retinene$_2$) liver storage of vitamin A$_2$ was roughly in proportion to the dose.

Rather large single doses of retinene$_2$ (130 μg.) and vitamin A$_2$ (120 μg.) promptly cured early xerophthalmia and restored growth, but quantitative biological assays were not carried out.

Replacement of vitamin A by vitamin A$_2$. Three albino rats 6 weeks old and weighing about 90 g. each were placed on a vitamin A-free diet. All the animals grew slowly, but from the 13th to the 15th week weights were stationary or falling. Each rat was then given retinene$_2$ (about 33 μg.) in arachis oil twice a week orally by means of a syringe. Growth was promptly resumed and continued slowly for the next 3 months. Dosage was discontinued from the 22nd to the 24th week and then resumed, but there was no break in growth. At the age of 36 weeks the rats were kept in the dark for 18 hr., anaesthetized in a red light and killed by drawing blood (cardiac puncture) into a syringe previously moistened with potassium oxalate solution. The livers and kidneys of each rat were tested for vitamins A and the bulked blood was similarly assayed. Results are sumarized in Table 1. The six retinas were combined and it was not apparent on testing for rhodopsin that any detectable replacement of rhodopsin by porphyropsin had occurred.

Ponndorf reduction of retinene$_2$

Crystalline retinene$_2$ (5 mg., m.p. 77–78°, $E^{1\%}_{1\ cm.}$ 386 mμ., 1450) in *iso*propanol was refluxed with aluminium *iso*propoxide (0·3 g.) for 18 hr. on the water bath. The refluxing was twice interrupted after 5 hr. and the mixture kept at 0° overnight and at each stage the product was tested. When the colour-test maximum at 693 mμ. was shown strongly, the heating was discontinued. After cooling, the mixture was treated with KOH to decompose the alkoxides and extracted with light petroleum. After washing, the solvent was removed under suction. The dry residue was dissolved in light petroleum and poured onto a column (2 × 1·75 cm.) of weakened alumina (10% water, w/w). On development with light petroleum, unchanged retinene$_2$ (about 0·4 mg.) quickly passed through the column leaving a yellow adsorbed zone. Elution with ethanol gave vitamin A$_2$, free from substances showing interfering absorption. The yield of vitamin A$_2$ was approximately 50% of the retinene$_2$ which reacted. The spectrum of vitamin A$_2$ obtained in this way shows only one definite maximum in the region 270–295 mμ. and the curve with $\lambda_{max.}$ 351 and 286 mμ. agrees very well with that of Shantz (1948).

In another experiment 35 mg. of crystalline retinene$_2$ were subjected to reduction (Ponndorf) and the product worked up as before. After two chromatographic adsorptions an apparently homogeneous fraction containing about 15 mg. vitamin A$_2$ was obtained. This was dissolved in light petroleum (2 ml.) and left at −70° to crystallize. After 2 months, supernatant liquor was decanted from a small amount of solid. A little fresh solvent was added but the vitamin A$_2$ would not crystallize. The solvent was then removed and the residue weighed carefully (about 3 mg.). The following figures were obtained: $E^{1\%}_{1\ cm.}$ 351 mμ., 1490 (light petroleum) and $E^{1\%}_{1\ cm.}$ 693 mμ. (colour test), 4000 (see Figs. 1 and 2). Although high accuracy is not claimed we regard these measurements as trustworthy within a few per cent.

Yields of vitamin A$_2$ from retinene$_2$ by this process are not, however, satisfactory.

Table 1. *Vitamins A$_1$ and A$_2$ in rats given retinene$_2$ (0·33 μg. twice weekly) for 3 months*

Rat no.	Weight (g.)			Vitamin A$_1$ and A$_2$ content (i.u./g.)				Volume (ml.)		Vitamins A$_1$ and A$_2$ in i.u. (1 ml. serum)*	
				Liver		Kidney					
	Body	Liver	Kidney	A$_1$	A$_2$	A$_1$	A$_2$	Blood	Serum	A$_1$	A$_2$
1	250	10·9	2·3	115	52	8·6	3·6	9·5	4·6		
2	218	7·2	1·8	4	5	14·0	8·5	7·5	3·3	1·83	0·95
3	275	9·7	2·3	10	15	13·7	9·8	9·5	4·4		

* Combined sample.

Reduction of retinene$_2$ by $LiAlH_4$

Lithium aluminium hydride (200 mg.) was finely ground and dissolved in anhydrous diethyl ether (20 ml.). Crystalline retinene$_2$ (50 mg.) was also dissolved in dry ether (50 ml.). The reagent was added slowly with stirring at 0°. The solution quickly became pale yellow and then almost colourless (4–5 min., protected against strong light). (If the solution is left for a longer time it turns violet and the yield of vitamin A_2 is much reduced.) The excess $LiAlH_4$ was decomposed by dropwise addition of water, the flask being cooled by means of ice water. The mixture was extracted with redistilled ether and the ethereal layer washed and then dried (Na_2SO_4). The solvent was removed under reduced pressure. The residue (30 mg., $E^{1\%}_{1\,cm.}$ 351 mμ., 970) was mainly vitamin A_2 (693 mμ. peak in colour test). This was chromatographed on alumina weakened by addition of water (10%, w/w). A very small fraction showing maxima at 350 and 285 mμ. with an inflexion at 275 mμ. was carried through by light petroleum (neovitamin A_2?). This was followed by a fraction showing maxima at 320 and 345–350 mμ. Development with light petroleum-diethyl ether (95/5, v/v) gave a vitamin A_2 fraction (15 mg.). Doubling the proportion of ether resulted in elution of a small amount of oxidized material and anhydrovitamin A_2. Other oxidation products were eluted by pure ethanol. Rechromatographing the vitamin A_2 fraction gave a fraction eluted by

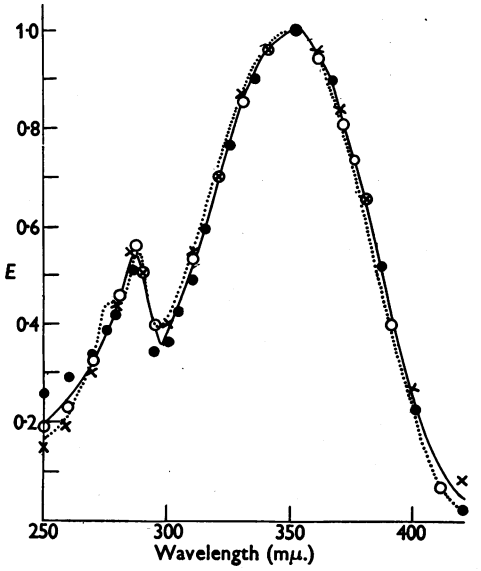


Fig. 1. Absorption spectrum of vitamin A_2 in ethanol, ——; preparations obtained by reduction of retinene$_2$ *in vivo*, ●; *in vitro* (Ponndorf), ○; ($LiAlH_4$), · · · · ·; vitamin A_2 (from Shantz, 1948), ×.

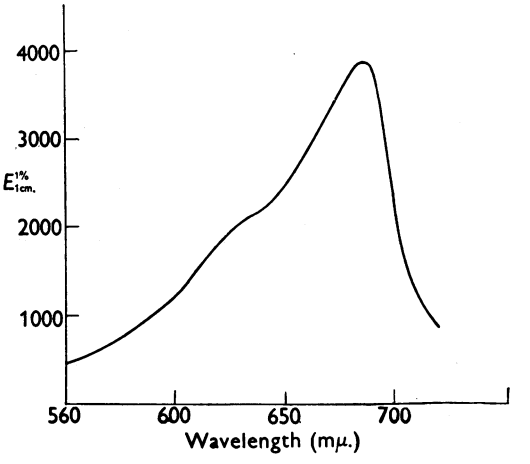


Fig. 2. Absorption curve on the blue solution obtained by the interaction of vitamin A_2 and the $SbCl_3$ reagent.

Table 2. *Spectroscopic properties of vitamin* A_2

Solvent	$\lambda_{max.}$ (mμ.)	$E^{1\%}_{1\,cm.}$	Inflexion (mμ.)	$E_{287\,m\mu}/E_{350\,m\mu}$
Ethanol*	352	1460	None	0·55
	287	820	None	—
Ethanol†	352	1330	None	0·51
	288	678	None	—
Ethanol‡	351	1410	277	$0{\cdot}54_5$
	286	698	—	—
Light petroleum‡	348	1390	277	0·54
	286	750	—	—
*cyclo*Hexane‡	351·5	1320	275	$0{\cdot}55_5$
	287	733	—	—
*iso*Propanol‡	351	1370	277	0·55
	285	753	—	—
Chloroform‡	356·5	1280	282	0·55
	291·5	704	—	—
$SbCl_3$ colour test*	693	4100	—	—
$SbCl_3$ colour test†	693	3700	—	—
$SbCl_3$ colour test‡	693	3870	—	—

* Shantz (1948). † Farrar, Hamlet, Henbest & Jones (1951). ‡ Present work.

5% ether-light petroleum, which in *cyclo*hexane showed the 350 and 286 mμ. maxima and a barely perceptible step-out at 275 mμ. (see Fig. 1).

The properties of vitamin A$_2$ are shown in Table 2.

Preparation of anhydrovitamin A$_2$

To about 8 mg. of vitamin A$_2$ were added 15 ml. approx. 0·03 N-ethanolic HCl (anhydrous). The solution was left to stand at room temperature in the dark for 15 min. The greenish solution was neutralized, extracted with redistilled ether and finally chromatographed.

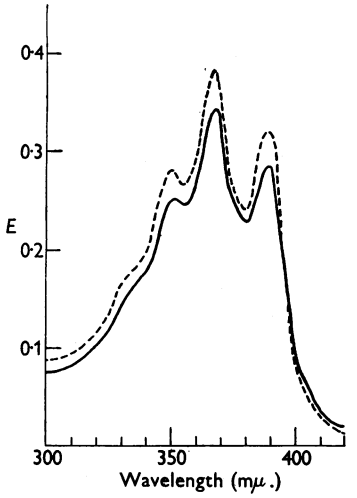


Fig. 3. Absorption spectrum of anhydrovitamin A$_2$ in ethanol, ——; and in light petroleum, - - - - -. (Absolute values not certain.)

The absorption spectrum (Fig. 3) is entirely characteristic, but the product differs from anhydrovitamin A in giving a 693 mμ. band in the colour test as compared with 620 mμ.

DISCUSSION

There is little hope of isolating sufficient retinene$_2$ from retinas for more than spectroscopic characterization. Proof that Wald's retinene$_2$ is the aldehyde of vitamin A$_2$ therefore rests on cumulative evidence which must include precise information about vitamin A$_2$ itself.

Karrer & Bretscher (1942) and Karrer, Bretscher & Geiger (1943) prepared from pike-liver oil a vitamin A$_2$ concentrate with $\lambda_{max.}$ 345 mμ. and $E^{1\%}_{1\,cm.}$ 1450. This was probably impure. Shantz (1948) observed $\lambda_{max.}$ 352 mμ. $E^{1\%}_{1\,cm.}$ 1460 (ethanol) and $\lambda_{max.}$ 287 mμ. $E^{1\%}_{1\,cm.}$ 820, with $\lambda_{max.}$ 693 mμ. $E^{1\%}_{1\,cm.}$ 4100 in the colour test. Although vitamin A$_2$ has itself never yet been obtained crystalline, its *p*-phenylazobenzoate (m.p. 76–77°) has been crystallized. On the basis of the open chain (ψ-ionone) formula advocated earlier (Karrer, Geiger & Bretscher, 1941), the empirical formula would be $C_{33}H_{38}O_2N_2$. (Required C, 80·1; H, 7·7; N, 5·7%. Found: C, 80·1; H, 7·8; N, 5·9%.) This agreement implies that vitamin A$_2$ is isomeric with vitamin A$_1$ ($C_{20}H_{30}O$). Anhydrovitamin A$_2$ (m.p. 89·5°) with seven double bonds apparently corresponded with $C_{20}H_{28}$ (open-chain formula). (Required C, 89·5; H, 10·5%. Found: C, 89·2; H, 10·7%.) Against this, if retinene$_2$ is $C_{20}H_{26}O$ (Cama *et al.* 1952), it is difficult to consider any formula but $C_{20}H_{28}O$ for vitamin A$_2$, and $C_{20}H_{26}$ for anhydrovitamin A$_2$.

Karrer & Schneider (1950) re-investigated vitamin A$_2$ *p*-phenylazobenzoate and raised the melting point to 94–95° but lacked material for further recrystallization. The rise in melting point from 76–77° (Shantz) to 94–95° may mean that the preparations were isomeric (cf. Farrer, Hamlet, Henbest & Jones, 1951). $C_{33}H_{38}O_2N_2$ (mol.wt. 494·3) requires C, 80·1; H, 7·7%; whereas $C_{33}H_{33}O_2N_2$ (mol.wt. 492·3) requires C, 80·4; H, 7·3%. (Found: C, 80; H, 7·2%.) As the analyses were not quite decisive, Karrer & Schneider (1950) treated vitamin A$_2$ (recovered from the *p*-phenylazobenzoate) with ozone and failed to obtain acetone, although they had no difficulty in detecting it in the ozonization products of ψ-ionone and ψ-ionylidene-ethanol. This result seems to exclude the open-chain formula $C_{20}H_{30}O$ for vitamin A$_2$ and, by implication, $C_{20}H_{28}O$ for retinene$_2$.

Farrar *et al.* (1951) have synthesized vitamin A$_2$ via 3-dehydrovitamin A$_1$ acid, and their 3-dehydrovitamin A$_1$ cannot be distinguished from vitamin A$_2$. Moreover, their retinene$_2$ prepared by the method of Ball, Goodwin & Morton (1948) (MnO_2 oxidation) is identical with our crystalline retinene$_2$. The spectroscopic characterization will be discussed later.

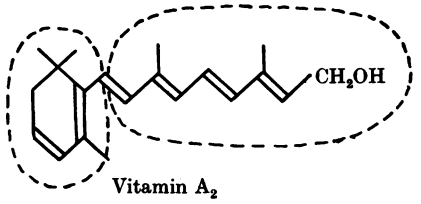
Now vitamin A$_2$ is biologically active. It is generally agreed that it can (at least partially) act as a substitute for vitamin A$_1$ in rats and there is no reason to doubt its biological activity in all species where it occurs naturally. Shantz & Brinkman (1950) have shown that, for rats, spectroscopically pure vitamin A$_2$ possesses about 40% of the potency of an equal weight of vitamin A$_1$. The biogenesis of vitamin A$_2$ remains obscure, but from the work of Morton & Creed (1939) perch can use β-carotene as a vitamin A$_2$ precursor. It is not clear whether vitamin A$_1$ must be made first, i.e. whether or not dehydrogenation precedes cleavage of the C_{40} compound. All the available evidence indicates that unless a carotenoid contains one intact β-ionone ring it cannot act as a provitamin A$_1$. This would, of course, exclude lycopene, with an open-chain structure.

The interesting work of Meunier, Jouanneteau & Zwingelstein (1950) and Meunier (1951) shows that manganese dioxide can be used effectively to split the β-carotene molecule and yield retinene$_1$. This

finding is in harmony with other work (Goss & McFarlane, 1947; Wendler, Rosenblum & Tishler, 1950) and serves to emphasize the versatility of manganese dioxide as an oxidizing agent simulating the action of dehydrogenases *in vivo*.

The next step in the work of Meunier (1951), however, requires very careful scrutiny. Lycopene undergoes fission with manganese dioxide in a manner analogous to β-carotene to give an aldehyde said to be retinene$_2$ and to be reducible to vitamin A$_2$. Biological tests are described which indicate that the lycopene fission product is growth-promoting to rats on a vitamin A-deficient diet at a dose level of 6 μg./day. From these results it is argued that Karrer's original open-chain formula for vitamin A$_2$ is correct.

This work raises difficult questions. If it can be confirmed, the absence of biological activity for lycopene itself is difficult to understand. In the unlikely event that Karrer's structure for lycopene (Karrer & Jucker, 1948) is incorrect, Meunier's aldehyde might be retinene$_2$, but the inactivity of lycopene would be even more puzzling. To compel assent the French workers need to demonstrate the purity of the lycopene used; they give m.p. 176°, $\lambda_{max.}$ 485 mμ., $E^{1\%}_{1\,cm.}$ 1950 ($CHCl_3$) (Meunier, 1951), whereas Zechmeister, Le Rosen, Schroeder, Polgar & Pauling (1943) give m.p. 175°, $\lambda_{max.}$ 474 mμ., $E^{1\%}_{1\,cm.}$ 3480. If the $E^{1\%}_{1\,cm.}$ values found by Zechmeister *et al.* are correct the purity of the lycopene used by Meunier was only 66 %, but the melting point argues against such a suggestion.



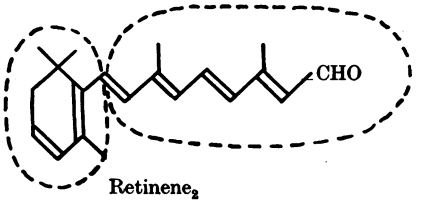
Vitamin A$_2$

Meunier also needs to demonstrate that his aldehyde is identical with retinene$_2$. As we have shown, $\lambda_{max.}$ in the colour test is 730–740 mμ. fading to 705 mμ. Meunier *et al.* record 715 mμ. The ultraviolet absorption should show $\lambda_{max.}$ 408 mμ. with an inflexion near 315 mμ. ($CHCl_3$). No information is given to suggest that this inflexion occurs in the aldehyde from lycopene.

At this stage it is necessary to discuss the spectroscopic properties of vitamin A$_2$. There is no doubt that the subsidiary absorption peak at 286 mμ. must be accepted as no less characteristic of vitamin A$_2$ than the main peak at 351 mμ. In fact, constancy of the ratio $E_{351\,m\mu}/E_{286\,m\mu}$ is a good criterion of purity (see Table 2). The absorption curve for the reduction product of the aldehyde derived from lycopene shows $\lambda_{max.}$ 352 mμ. in ethanol, but no mention is made of a 286 mμ. maximum (Meunier, 1951). The 693 mμ. absorption band in the colour test characteristic of vitamin A$_2$ also appears (690 mμ.) in Meunier's product. He does not, however, give E values, and although the aldehyde from lycopene is very like retinene$_2$, the evidence for identity falls considerably short of proof.

It cannot be doubted that the case in favour of the open-chain formula has not been made out. It is of course possible that the rat may be able to close a ring from the ψ-ionone part of the lycopene molecule, but this is not part of the argument advanced by Meunier although it could account for the biological activity. If so, however, the question inevitably recurs, why is the rat unable to use lycopene?

The spectroscopic properties of retinene$_2$ and vitamin A$_2$ could not, however, have been predicted from those of retinene$_1$ and vitamin A$_1$. The displacement of $\lambda_{max.}$ (326–351 mμ., A$_1 \rightarrow$ A$_2$) is qualitatively and perhaps quantitatively consistent with the introduction of an extra conjugated bond, but from general experience on polyene spectra such a change should be accompanied by a rise in $\epsilon_{max.}$ when electron mobility is unhindered. Actually $\epsilon_{max.}$ falls from about 50 000 to about 38 000. Both vitamin A$_2$ and retinene$_2$ have unexpectedly low $\epsilon_{max.}$ values. In the case of vitamin A$_2$ the fall in $\epsilon_{max.}$ is accompanied by the appearance of the subsidiary sharp absorption peak at 286 mμ.



Retinene$_2$

This means that the observed absorption curve is made up of three contributions: (*a*) that due to six conjugated double bonds ($\lambda_{max.}$ 351 mμ.); (*b*) that due to a proportion of the solute molecules in which less than six (perhaps two) conjugated double bonds give rise to the 286 mμ. band, i.e. due to a molecular 'partial'; (*c*) that due to the remaining molecular 'partial' with perhaps four conjugated double bonds. (For a discussion of 'bands of partial oscillation' see Lewis & Calvin (1933) and MacColl (1947).)

The ring structure alone might well give rise to the 286 mμ. band (cf. 7-dehydrocholesterol with peaks at 270, 281·5 and 293·5 mμ.) but, if so, a second band at 300–310 mμ. would certainly be expected for the other 'partial'. No such peak is observed in vitamin A$_2$, but it could be masked by

overlapping contributions from the (*a*) and (*b*) absorptions.

The displacement of $\lambda_{max.}$ in retinene$_2$ (vitamin A$_2$ → retinene$_2$; 351 → 385 mμ.) can be ascribed to the —CHO group conjugated to the main system, but the lack in retinene$_2$ of a sharp secondary peak to correspond with the 286 mμ. of vitamin A$_2$ is not easy to explain. There is a definite inflexion near 310 mμ., but neither the intensity nor the poor definition is consistent with the probability of 'partials' being the same as in vitamin A$_2$.

The small band near 275 mμ. shown by vitamin A$_2$ preparations is puzzling. It appears very regularly in certain freshwater fish liver oils and their unsaponifiable fractions rich in A$_2$. This is particularly true for Nile fishes (Salah, private communication). We have obtained some preparations (by Ponndorf reduction of retinene$_2$) in which the inflexion was not noticeable, but all our most recent reduction products ($LiAlH_4$) from purified retinene$_2$ show the inflexion even after careful chromatography of the product. On the other hand, the best preparations of Farrar *et al.* (1951) do not show the inflexion (private communication from Prof. E. R. H. Jones) nor do those of Shantz (1948). At present it is difficult to assert that the inflexion is due to an impurity rather than a vitamin A$_2$ isomer and the matter must be left open.

The vitamin A$_2$ obtained by reduction of retinene$_2$ shows the $SbCl_3$ colour-test peak at 693 mμ. ($E^{1\%}_{1\,cm.}$ about 4000) with a weak inflexion near 640 mμ. (Fig. 2). Most workers on vitamin A$_2$ have observed this inflexion, but it is possible that when vitamin A$_2$ is obtained as a single isomer it may be absent (cf. Shantz, 1948) just as the secondary colour-test selective absorption at 580 mμ. is not shown by the purest *all-trans* vitamin A$_1$ (Cama, Collins & Morton, 1951).

The vitamin A$_2$ obtained in the present work forms anhydrovitamin A$_2$ smoothly and the product shows the expected spectrum (Fig. 3). The fact that anhydrovitamins A$_1$ and A$_2$ show identical absorption maxima, but differ in respect of their colour-test maxima, is confirmed. Plausible structures have been advanced by various groups of workers, but objections can be raised to all of them and in our view new evidence is needed before the matter can be settled.

SUMMARY

1. When retinene$_2$ is fed to avitaminotic rats receiving a diet free from carotenoids or vitamin A, it is converted to vitamin A$_2$ which can be found in the liver within 24 hr. The recovered vitamin A$_2$ shows an absorption spectrum agreeing very well with that of the purest preparations.

2. Vitamin A$_2$ is moderately well stored by rats but is not a wholly satisfactory substitute for vitamin A$_1$.

3. Reduction of retinene$_2$ by the Ponndorf and lithium aluminium hydride methods results in vitamin A$_2$, the product being indistinguishable from the purest natural vitamin A$_2$ preparations.

4. The $SbCl_3$ colour reaction of vitamin A$_2$ has been studied in detail.

5. The evidence is very strong that vitamin A$_2$ is 3-dehydrovitamin A$_1$.

We are indebted to the Medical Research Council for financial assistance.

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