Studies in Vitamin A

22. OPPENAUER OXIDATION OF VITAMIN A₁

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The merit of the Oppenauer oxidation (Oppenauer, 1937; reviewed by Djerassi, 1951) using aluminium alkoxides and a hydrogen acceptor, is that unsaturated alcohols can be converted to aldehydes and ketones without affecting the ethylenic linkages. Applied to vitamin A alcohol it ought to yield retinene₁ (vitamin A₁ aldehyde) as the primary product. Hawkins & Hunter (1944*a*), using aluminium isopropoxide and acetaldehyde as hydrogen acceptor, have in fact obtained retinene₁. There is, however, a marked tendency for secondary reactions to occur and so to diminish the yield of the initial dehydrogenated product. Haworth, Heilbron Jones, Morrison & Polya (1939) varied the conditions, and in particular used aluminium *tert*-butoxide and diethyl ketone as hydrogen acceptor. They did not obtain retinene, but found instead that a crystalline oxime (isolated in low yield from the reaction mixture) corresponded with an 'aldehyde' C₂₀H₂₆O. At that time there was no suspicion that the retinenes were the aldehydes of vitamins A₁ and A₂ and for a time the observation attracted no special interest.

The tentative structure

assigned to the C₂₀ aldehyde implied dehydrogenation in the β-ionone ring as well as at the primary alcohol grouping. This structure was later attributed to retinene₂ (Morton, Salah & Stubbs, 1947) largely on the basis of the properties of crystalline vitamin A₂ aldehyde prepared from natural vitamin A₂.

Synthetic retinene₂ has recently been prepared (Farrar, Hamlet, Henbest & Jones, 1951) and its properties agree very well with those of the retinene₂ prepared from vitamin A₂ of natural origin (Cama *et al.* 1952*b*).

The C₂₀ aldehyde obtained by Haworth *et al.* (1939) was not fully characterized, except through the oxime, and it was clearly necessary to repeat the work, aiming at isolation and characterization of the aldehyde itself. This has proved to be a rather

difficult matter because the Oppenauer product is always heterogeneous.

It will be shown that the C₂₀H₂₆O preparation can be reproduced, and that the substance very closely resembles retinene₂ but is actually a totally distinct compound, the precise structure of which remains uncertain.

EXPERIMENTAL

Materials

Vitamin A concentrates. (1) Much of our earlier work was carried out on partially sterol-free unsaponifiable fractions from high-potency Portuguese tunny-liver oils. (2) Later, many experiments were made using similar fractions from ester concentrates. The unsaponifiable fractions, after crystallizing out much sterol, usually contained 50–55% vitamin A. (3) Finally, synthetic vitamin A acetate (Roche) was used after careful saponification. Some experiments were carried out on the acetate itself.

Reagents. Aluminium *tert*-butoxide was prepared by the method of Wayne & Adkins (1941). Aluminium isopropoxide was prepared according to Wilds (1944). Benzene was dried over Na wire and redistilled before use. Diethyl ketone (commercially pure) was dried over anhydrous CuSO₄, filtered and distilled.

Oppenauer reaction

The following experiment was typical. An all-glass refluxing apparatus was used and reactants were added in the following order: vitamin A concentrate (equivalent to 6 g. vitamin A), diethyl ketone (30 ml.), benzene (200 ml.) and aluminium isopropoxide (7.3 g.). The mixture was gently refluxed continuously (48 hr.). From the dark-red reaction mixture two small equal portions were taken and the aluminium alkoxides were decomposed in one by means of water and in the other by 10% H₂SO₄ (v/v). Light petroleum extracts from the two experiments could not be distinguished spectroscopically, and it was accepted that the acid had no deleterious effect. The main portion was then treated with excess of 10% H₂SO₄ (little emulsification occurs with acid) and light petroleum was added. After washing with NaHCO₃ solution and water the extract was dried (Na₂SO₄) and the solvent evaporated completely under reduced pressure. The residue was dissolved in light petroleum (50 ml.) and chromatographed on alumina (Peter Spence and Co., Grade O, weakened by addition of water, 10% w/w). The main fraction 2 (Table 1) was rechromatographed on alumina containing 8% water and a fraction (2*d*) was retained and rechromatographed over

alumina containing 11% water to give two distinct fractions, $2d_{5-9}$ and $2d_{10-15}$, which were separately crystallized at low temperature (-60°) from light petroleum. After three crystallizations the two products were analysed.

From Table 2 it will be seen that the less strongly adsorbed compound is probably $C_{20}H_{26}O$. It gives an oxime of m.p. $175-177^\circ$ agreeing with that recorded by Haworth *et al.* (1939), Hawkins & Hunter (1944b) and Hunter (1950).

Table 1. *Properties of fractions obtained by chromatography*

Fraction	Wt. (g.)	Absorption maxima (m μ .)	SbCl ₅ colour-test maxima (m μ .)
Stage 1. Alumina containing 10% water			
1 (yellow)	0.19	355, 370, 390, 415	715, 620
2 (dark red)	8.0	375, 390	715-720
3 (yellow)	0.37	330	710 weak, 620 strong
4 (yellow)	0.13	330	620
5* (red)	1.7	310	620
Stage 2. Alumina containing 8% water			
2a (yellow)	—	270, 350, 370, 390	620
2b (yellow)	—	270, 350, 370, 390	620
2c (red)	—	330, 350, 365, 390	710
2d (red)	—	385	715
2e* (red)	—	330	640
Stage 3. Alumina containing 11% water			
$2d_{1-2}$	—	380	695-700 $E_{1\text{cm}}^{1\%}$ 1170
$2d_{3-4}$	—	385	~685
$2d_{5-9}$	—	385	720 $E_{1\text{cm}}^{1\%}$ 1085 (640 weak)
$2d_{10-15}$	—	385	720 $E_{1\text{cm}}^{1\%}$ 1050
$2d_{16}^*$	—	375-385	640-715

* Eluted by means of ether.

Table 2. *Analytical results and spectroscopic properties of $C_{20}H_{26}O$ compound obtained by Oppenauer oxidation of vitamin A (m.p. $79-80^\circ$)*

(a)				
	C (%)	H (%)	O (%)	H/C (atoms)
Found	84.93	9.38	5.69	1.31
$C_{20}H_{26}O$ requires	85.04	9.29	5.66	1.3
$C_{20}H_{28}O$ requires	84.5	9.86	5.64	1.4
(b)				
Solvent	$\lambda_{\text{max.}}$ (m μ .)	$E_{1\text{cm}}^{1\%}$	Inflexion (m μ .)	
Light petroleum	386	1690	270	
cycloHexane	390	1740	270	
Ethanol	395	1450	280	
Chloroform	406	1480	280	
SbCl ₅ colour test	720	—	—

Fig. 1. Absorption spectrum of $C_{20}H_{26}O$ compound in light petroleum, $\bullet$ — $\bullet$; cyclohexane, ---; ethanol,; and chloroform, —.

Table 3. *Properties of the oxime of the $C_{20}H_{26}O$ compound (m.p. $175-177^\circ$)*

(i)			
	C (%)	H (%)	N (%)
$C_{20}H_{27}ON$ requires	80.8	9.2	4.7
$C_{20}H_{29}ON$ requires	80.2	9.8	4.7
Found	81.14	9.57	4.65 (a)
	80.6	9.8	4.6 (b)
	80.5	10.0	4.5 (b)
	80.1	9.55	4.5 (c)
	80.2	9.75	4.8 (c)
(ii)			
	(a)	(c)	
$\lambda_{\text{max.}}$ (light petroleum)	375 m μ .	380 m μ .	
$E_{1\text{cm}}^{1\%}$	1890	1940*	

(a) Present work.

(b) Haworth *et al.* (1939).

(c) Hawkins & Hunter (1944b).

* Misprint in original paper.

Fig. 2. Absorption spectrum of $C_{20}H_{26}O$ -2:4-dinitrophenylhydrazone in chloroform,; $C_{20}H_{26}O$ -semicarbazone in chloroform, —; and $C_{20}H_{26}O$ -oxime in light petroleum, ---.

The $C_{25}H_{36}O$ compound (0.15 g.) was dissolved in ethanol, and hydroxylamine hydrochloride (0.1 g.) and sodium acetate (0.5 g.) were added in a small volume of water (5 or 6 drops). The mixture was refluxed (2 hr.) under N_2 , cooled, poured into water (250 ml.) and left to stand at 0° overnight. The flocculent yellow precipitate was filtered off and re-crystallized from light petroleum. The properties of the oxime are listed in Table 3.

Table 4. *Analytical results and spectroscopic properties of C_{25} ketone obtained by Oppenauer oxidation of vitamin A in presence of diethyl ketone (m.p. $76-78^\circ$)*

	(a)		
	C	H	O
	(%)	(%)	(%)
Found (present work)	85.27	9.66	5.07
(Jones, 1951)	85.6	10.6	3.8
$C_{25}H_{36}O$ requires	85.15	10.3	4.65
$C_{25}H_{34}O$ requires	85.65	9.7	4.65

	(b)		
	λ_{max}	$E_{1\%}^{1cm}$	Inflexion
	($m\mu$.)		($m\mu$.)
Solvent			
Light petroleum	390	1354	270
cyclohexane	390	1415	275
Ethanol	400	1271	275
Chloroform	408	1223	280
$SbCl_3$ colour test	720, 640	—	—

Fig. 3. Absorption spectrum of $C_{25}H_{36}O$ compound in light petroleum, $\bullet-\bullet$; cyclohexane, ---; ethanol,; and chloroform, —.

The 2:4-dinitrophenylhydrazone (m.p. $160-162^\circ$) showed λ_{max} $455 m\mu$, $E_{1\%}^{1cm}$ 1050 ($CHCl_3$). The semicarbazone m.p. 152° , showed λ_{max} $390 m\mu$ (EtOH) and $400 m\mu$ ($CHCl_3$) with $E_{1\%}^{1cm}$ 1860.

The second crystalline product (from $2d_{10-15}$) showed m.p. $76-78^\circ$ (see Table 4).

By using aluminium *tert.*-butoxide (and diethyl ketone) the product is a mixture which contains either a C_{20} compound (Haworth *et al.* 1939; Hawkins & Hunter, 1944b) or a C_{25} ketone (Jones, 1951) or possibly both. In one or two of our experiments we obtained the former compound, but in most cases the only crystalline material which could be isolated after repeated chromatography was a C_{25} ketone of m.p. $79-80^\circ$. This showed λ_{max} $390 m\mu$ and $E_{1\%}^{1cm}$ 1750–1800 (cyclohexane) (Fig. 3). The position of the peak agrees closely with that of retinene₂ and that of the $C_{20}H_{36}O$ com-

pound, but the absorption curve is narrower and ϵ_{max} distinctly higher. The subsidiary maximum at $310 m\mu$ of retinene₂ is entirely absent.

Oppenauer oxidation of retinene₁

If the $C_{20}H_{36}O$ compound is derived from vitamin A by a two-stage dehydrogenation, retinene₁ is the likely intermediate. Accordingly, retinene₁ (1.0 g. cryst.) was refluxed continuously (48 hr.) with diethyl ketone (2 ml.), benzene (75 ml.) and aluminium isopropoxide (1.5 g.). The product was worked up as before. A compound of m.p. 77° was isolated, λ_{max} $387 m\mu$, $E_{1\%}^{1cm}$ 1700 (cyclohexane), $396 m\mu$, $E_{1\%}^{1cm}$ 1610 (EtOH); 2:4-dinitrophenylhydrazone, m.p. 162° . The compound agreed with the $C_{20}H_{36}O$ material already described.

Oppenauer oxidation of vitamin A acetate

If it were possible in the reaction to dehydrogenate in the β -ionone ring, vitamin A₂ acetate or vitamin A₂ might have been formed and detected by the ultraviolet absorption maxima at 350 and $285 m\mu$ or by the $SbCl_3$ colour-test maximum at $693 m\mu$. In no case out of many trials on vitamin A acetate or natural esters was any evidence found for this change. The reaction products showed intense selective absorption with maxima at 415, 390, 370 and $350 m\mu$, reminiscent of anhydrovitamin A₁ or A₂, but not identical with either, since these substances do not show a $415 m\mu$ peak. The absorbing materials readily underwent polymerization to very pale amorphous solids which could not be crystallized.

Fig. 4. Absorption spectrum of $C_{20}H_{36}O$ compound in light petroleum, —; and reduction product ($LiAlH_4$) in light petroleum, ---. $E_{1\%}^{1cm}$ max. (at $386 m\mu$.) 1690.

Reduction of $C_{20}H_{36}O$ compound with lithium aluminium hydride

The compound was readily reduced to an alcohol with a flat peak maximum at $350-360 m\mu$ (ϵ value lower than that of the aldehyde, cf. Fig. 4). A band in this position is characteristic of vitamin A₂ but the secondary peak at $286 m\mu$ shown by the vitamin is absent (cf. Cama, Dalvi, Morton & Salah, 1952a). Although the reduction product is similar to vitamin A₂ it is clearly a distinct substance. This is borne out by the fact that its $SbCl_3$ colour-test maximum is at $720 m\mu$ (instead of $693 m\mu$.) and also by the failure to obtain from it anhydrovitamin A₂ (λ_{max} 393, 370, $350 m\mu$, $SbCl_3$ colour test $693 m\mu$.)

Reduction of C₂₀H₂₆O compound in vivo

The crystalline compound was dissolved in diethyl ether, and small portions were added to arachis oil, and the ether removed by a current of N₂. Young newly weaned rats having low vitamin A reserves were given (orally, by syringe) arachis oil containing 20, 50 and 100 μg. of the C₂₀ compound. The animals were killed by CHCl₃ 24 hr. later and the livers were ground with silver sand and anhydrous Na₂SO₄. The lipids were extracted with diethyl ether and the solvent removed after filtering. The residue was examined for ultraviolet absorption and by means of the SbCl₃ colour test.

On no occasion was there any evidence of the formation of vitamin A₂. The C₂₀ compound had undoubtedly been absorbed and probably reduced because the 720 mμ. colour-test peak was shown by the liver extracts and the 385 mμ. band in the ultraviolet absorption had disappeared.

DISCUSSION

A compound C₂₀H₂₆O (not crystallized) had been claimed by Haworth *et al.* (1939) to be an Oppenauer oxidation product of vitamin A on the basis of the properties of its oxime, m.p. 175–177°. Hawkins & Hunter (1944b) and Hunter (1950) clearly obtained the same oxime (m.p. 175–177°) by a similar route. So far as qualitative absorption spectra (λ_{max}. 385 mμ.) and the SbCl₃ colour test (λ_{max}. 730–735 mμ.) were concerned the C₂₀H₂₆O compound might well have been retinene₂ (Morton *et al.* 1947), but Ponndorf reduction (Hawkins & Hunter, 1944b) did not give vitamin A₂ as it should have done on that basis. Instead, an alcohol (λ_{max}. 350–370 mμ. SbCl₃ colour test 722 mμ.) was obtained and did not yield an anhydro compound analogous to anhydrovitamin A₂. As the structure attributed to the C₂₀H₂₆O compound was that since attributed to retinene₂ (Morton *et al.* 1947) and established by Farrar *et al.* (1951) it was necessary to obtain the Oppenauer oxidation product pure. When crystallized (m.p. 77–78°) the material analysed correctly for C₂₀H₂₆O and gave the oxime (m.p. 175–177°) obtained by previous workers (Table 2 and 3). The absorption peak (C₂₀H₂₆O) at 385 mμ. agreed with that of retinene₂, but the subsidiary maximum of the latter at 310 mμ. was lacking. The SbCl₃ colour test (λ_{max}. 720 mμ.) did not agree with that of retinene₂ (735–705 mμ.) and on reduction *in vitro* and *in vivo* the distinction between the product and vitamin A₂ is definite.

This makes it very improbable that in the Oppenauer oxidation the second stage of dehydrogenation takes place in the β-ionone ring. The failure to obtain vitamin A₂ by Oppenauer oxidation of vitamin A acetate is confirmatory. Since, however, retinene₁ yields the C₂₀H₂₆O compound, the first stage of dehydrogenation is the formation of vitamin A₁ aldehyde.

The structure of the C₂₀H₂₆O compound is quite uncertain. Among the possibilities are:

(a) being in our view the least likely. The unusual allene structures cannot, for lack of information on similar but simpler compounds, be justified on purely spectrophotometric evidence, although investigations of infrared or Raman spectra might prove useful (see Sheppard & Simpson, 1952).

The structure

belongs to retinene₂, but it is conceivable that a *cis*-isomer might be the C₂₀H₂₆O aldehyde. The main objection to this is that neither prolonged heating nor catalysis with iodine showed any evidence of rearrangement to give retinene₂. Similarly, the reduction product (LiAlH₄) failed to isomerize to vitamin A₂.

The C₂₅ ketone

is presumably a direct condensation product of retinene₁ and diethyl ketone. It is, however, also possible that the C₂₀H₂₆O compound may condense with diethyl ketone to give C₂₅H₃₄O. The former compound is quite easy to crystallize from light petroleum (m.p. 79–80°) and it shows a very high

$\epsilon_{\max.}$. If, however, a similar compound is obtained from a reaction mixture containing the $C_{20}H_{26}O$ compound its properties suggest $C_{25}H_{34}O$, i.e. it derives from the $C_{20}H_{26}O$ compound.

SUMMARY

1. Oppenauer oxidation of vitamin A using diethyl ketone as hydrogen acceptor results in a complex mixture from which a compound $C_{20}H_{26}O$ (isomeric with retinene₂) can be isolated. It gives an oxime, m.p. 175–177°, isolated by previous workers.

2. The $C_{20}H_{26}O$ compound, is reducible *in vitro* and *in vivo*, but the product differs from vitamin A₂.

3. The Oppenauer oxidation seems to proceed via retinene₁, $C_{20}H_{26}O$, which can either be further dehydrogenated to $C_{20}H_{24}O$ or can condense with diethyl ketone, to a ketone $C_{25}H_{36}O$. Condensation of the $C_{20}H_{26}O$ compound with diethyl ketone to yield $C_{25}H_{34}O$ is not excluded.

4. Vitamin A acetate is not dehydrogenated directly to vitamin A₂.

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The Structure of Ribonucleic Acids

1. CYCLIC NUCLEOTIDES PRODUCED BY RIBONUCLEASE AND BY ALKALINE HYDROLYSIS

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One of the most striking chemical differences between the two main types of nucleic acid is that *N*-sodium hydroxide or potassium hydroxide at room temperature hydrolyses ribonucleic acids (RNA) rapidly and completely to nucleotides, but does not hydrolyse deoxyribonucleic acids in this way. The nucleotides produced on alkaline hydrolysis of RNA were thought for many years to be nucleoside 3'-phosphates (Levene & Harris, 1932), but recently Carter (1950), Cohn (1950) and also Loring & Luthy (1951) have demonstrated that all four ribonucleotides exist in the alkaline hydrolysates as pairs of isomers. These pairs, which have been called 'a' and 'b' nucleotides, differ in their chromatographic behaviour and are quite distinct

from the nucleoside 5'-phosphates. They are stable to alkali, but may be interconverted by a short treatment with dilute acid (Brown, Haynes & Todd, 1950), and evidence has been given by Brown & Todd (1952*a*) that the 'a' and 'b' nucleotides are the nucleoside 2'- and 3'-phosphates, though not necessarily in that order, rather than α - and β -isomers (Doherty, 1950). The 'a' and 'b' nucleotides differ considerably in several properties such as solubility, and it is of interest to note that only the 'b' forms of the pyrimidine nucleotides are liberated from RNA by ribonuclease, while at pH 8 a crude phosphatase from potato acts much more rapidly on the 'b' form of adenylic acid than on the 'a' form (Cohn, 1951; Kornberg & Pricer, 1950).