30. *The Oxidation of Vitamin-A by the Oppenauer Reagent. Part II.*

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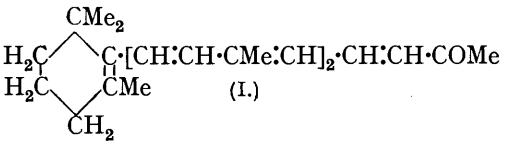
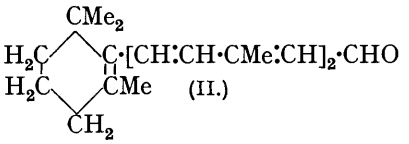
Oxidation of vitamin-A with aluminium *tert.*-butoxide in the presence of diethyl ketone gives rise to a C_{20} aldehyde, characterised by an *oxime*, m. p. 176—177°. Reduction of the aldehyde with aluminium *iso*propoxide gives an alcohol with an absorption maximum at 3590 A. in contrast to that of the original vitamin-A at 3280 A. It is believed that the aldehyde contains an additional double bond situated in the ring.

We have recently shown (J., 1938, 175) that the oxidation of a vitamin-A concentrate with aluminium *tert.*-butoxide in the presence of acetone gives a ketone, $C_{23}H_{32}O$ (I), formed by the condensation of the originally produced aldehyde with acetone. With the object of isolating the aldehyde itself, we have now investigated the reaction, using ketones with which condensation is either impossible or much more difficult to effect. Of the numerous compounds tried, including diethyl ketone, di*iso*propyl ketone, *p*-benzoquinone, *cyclo*hexanone, etc., only diethyl ketone proved satisfactory and attention has been concentrated on its use.

It was found that oxidation of a rich vitamin-A concentrate ($E^{1\%}_{1\,cm.}$ 3280 A. = 1350), obtained from a halibut visceral oil (Lovern, Edisbury, and Morton, *Nature*, 1937, **140**, 276), gave a crude product exhibiting an absorption band at 3960 A. ($E^{1\%}_{1\,cm.}$ = 690) together with that of unchanged vitamin-A ($E^{1\%}_{1\,cm.}$ approx. 400). By means of the Girard reagent P (Girard and Sandulesco, *Helv. Chim. Acta*, 1936, **19**, 1095) a carbonyl compound was isolated as an orange viscous oil showing a well-defined absorption maximum at 4010 A. ($E^{1\%}_{1\,cm.}$ = 1140), the extinction coefficient of which was raised to $E^{1\%}_{1\,cm.}$ = 1430 after chromatographic adsorption of the substance on calcium hydroxide. With chloroformic antimony trichloride it gave a blue-green colour exhibiting a single absorption band at 7400 A., in contrast to that at 6460 A. for the ketone (I). It readily formed a semicarbazone (not obtained quite pure) ($\lambda_{max.}$ 4030 A.) and a beautifully crystalline *oxime*, m. p. 176—177°, combustion analysis of which indicated that the parent compound possesses a C_{20}, and not a C_{25} formulation which would be expected if condensation involving diethyl ketone took place as in the case of acetone. This was confirmed by repeating the oxidation in

the presence of dipropyl ketone; although the yield was considerably smaller, the same oxime was then obtained, thus definitely establishing that the ketone simply acts as a


(I.)


(II.)

hydrogen acceptor. The reluctance of diethyl ketone to condense with the aldehyde is in agreement with the observation of Hibbert and Cannon (*J. Amer. Chem. Soc.*, 1924, **46**, 119) that, whereas citral condenses very readily with acetone in the presence of sodium ethoxide, no condensation takes place with diethyl ketone under similar conditions.

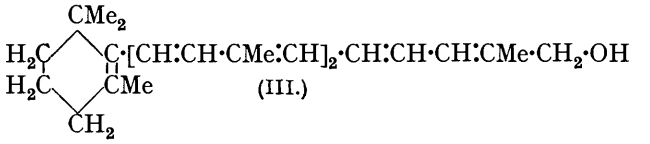
Biological tests show that the aldehyde, in contrast to the ketone (I), possesses growth-promoting properties and we have therefore attempted to regenerate the parent alcohol. Reduction with aluminium *iso*propoxide, however, failed to give vitamin-A, but a compound was formed exhibiting an absorption band at 3590 A. ($E^{1\%}_{1cm.} = 1300$) and giving with chloroformic antimony trichloride a blue-green colour ($\lambda_{max.}$ 7280 A.), in contrast to the blue colour shown by vitamin-A with absorption bands at 6170 and 5830 A. A Zerewitinov determination on the reduction product indicated that it contains one active hydrogen atom and preliminary feeding tests have shown it to be physiologically active.

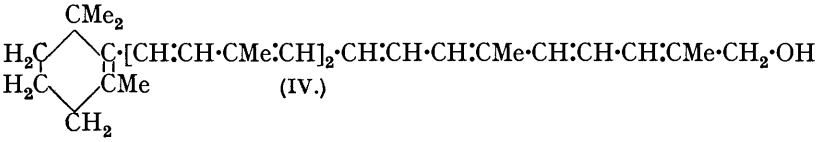
Two possible explanations of these results present themselves: either (a) the aldehyde is not the direct oxidation product, $C_{20}H_{28}O$, of vitamin-A, or (b) the Pondorff reduction is abnormal. From the spectrographic evidence the latter seems improbable, since, as shown in Table I, the extent of the displacement of the absorption band in the reduction product towards the shorter wave-lengths is in good agreement with that observed for other polyene carbonyl compounds. Turning to the alternative possibility, the position

TABLE I.

Carbonyl compound.	Conj. ⁼.	$\lambda_{max.}$, A.	Corresp. alcohol, A.	Displacement, A.
Octatrienal	3	3160	2700	460
Aldehyde from vitamin-A ...	?	4010	3590	420
α-Apo-2-carotenal	8	4790	4460	330
β-Apo-2-carotenal	9	4840	4530	310

of the absorption maximum (3590 A.) (Fig. 3) of the alcohol would appear to indicate that it contains one double bond more than vitamin-A, a conclusion which is strengthened

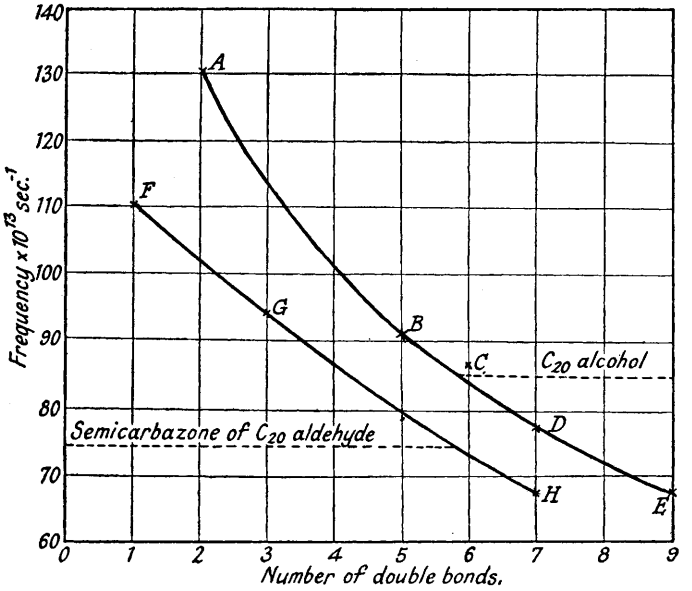

(III.)


(IV.)

by comparison with vitamin-A_2 (approx. 3500 A.) (Gillam, Heilbron, Jones, and Lederer, *Biochem. J.*, 1938, **32**, 405) and with the position (3560 A.) predicted by Karrer, Ruegger, and Geiger (*Helv. Chim. Acta*, 1938, **21**, 1171) for the latter compound from a comparison with vitamin-A (5 ⁼), β-apo-4-carotenol (7 ⁼) (III) and β-apo-2-carotenol (9 ⁼) (IV). Although these authors assume that there is a linear relationship between the wave-length maxima and the number of conjugated double bonds, it will be seen from Fig. 1 that the frequencies lie on a smooth curve in agreement with the findings of Hausser, Kuhn, and Seitz (*Z. physikal. Chem.*, 1935, **29**, *B*, 401) for the diphenyl polyenes. From the similar curve (Fig. 1) for the semicarbazones of β-*cyclo*citral, β-ionylideneacetaldehyde (Kuhn,

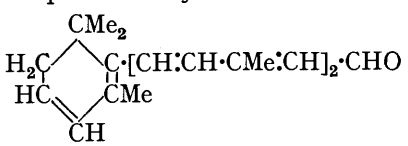
J., 1938, 613), and β-apo-4-carotenal (Karrer, Solmssen, and Gugelmann, *Helv. Chim. Acta*, 1937, **20**, 1020) it will be seen that the position of the absorption band of the semicarbazone of the C_{20} aldehyde also agrees with the presence of six conjugated ethylenic linkages, so that on the spectrographic evidence alone it would appear as though an additional double bond has been introduced during the oxidation. The only position

FIG. 1.

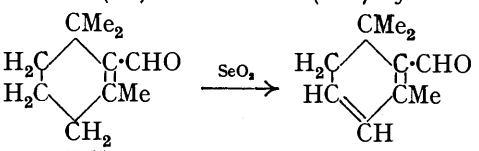


A. *β-Ional.*
B. *Vitamin-A.*
C. *Vitamin-A₂.*
D. *β-Apo-4-carotenol.*
E. *β-Apo-2-carotenol.*
F. *Semicarbazone of β-cyclocitral.*
G. *Semicarbazone of β-ionylideneacetaldehyde.*
H. *Semicarbazone of β-apo-4-carotenal.*

in which this could occur is in the β-ionone ring to give a compound of structure (V), a reaction paralleled by the conversion of β-*cyclo*citral (VI) into safranal (VII) by means of



(V.)



(VI.) (VII.)

selenium dioxide (Kuhn and Wendt, *Ber.*, 1936, **69**, 1549). Although so far we have only been able to carry out preliminary work with the object of confirming this explanation, we have found that ozonolysis of the aldehyde fails to give geronic acid, thus definitely establishing the absence of the β-ionone ring.

The likelihood that the aldehyde actually contains one double bond more than was expected raises the question as to the constitution of the C_{23} ketone (I) and in this connexion it is of interest to note that the position of its absorption maximum is identical with that of the aldehyde (Table II; Fig. 2), a result leading to the conclusion that the

TABLE II.

Compound.	$\lambda_{max.}$, A.	$\epsilon_{max.}$
(a) C_{23} Ketone (I)	4010	48,900
(b) C_{20} Aldehyde	4010	40,600
(c) Oxime of (b)	3860	73,500
(d) Semicarbazone of (a)	3970	86,000
(e) Crude ,, ,, (b)	4030	75,400

Solvent. ethyl alcohol.

ketone contains the normal β-ionone ring. It would thus seem that the oxidation proceeds to give the true vitamin-A aldehyde in the first place, which, in the presence of an unreactive ketone (diethyl ketone) as hydrogen acceptor, is further oxidised in the ring with loss of two hydrogen atoms. On the other hand, in the presence of acetone it apparently condenses immediately to give the ketone (I), which is not further oxidised, possibly owing to the presence of the water formed in the condensation. A partial confirmation of this reaction mechanism is supplied by an examination of the product formed by subsequent condensation of the aldehyde with acetone. If both the oxidation products contained the same ring system, the same C_{23} ketone should result. In actual fact this was not the case, for in the presence of either sodium ethoxide or aluminium *tert.*-butoxide a ketone was obtained which gave a p-*tolylsemicarbazone*, m. p. 206—207°, showing a depression on admixture with the *p*-tolylsemicarbazone of (I).

In the more detailed investigation which is now being actively pursued, it is our intention to examine the ozonolysis products of the aldehyde for the presence of αα-di-

FIG. 2.

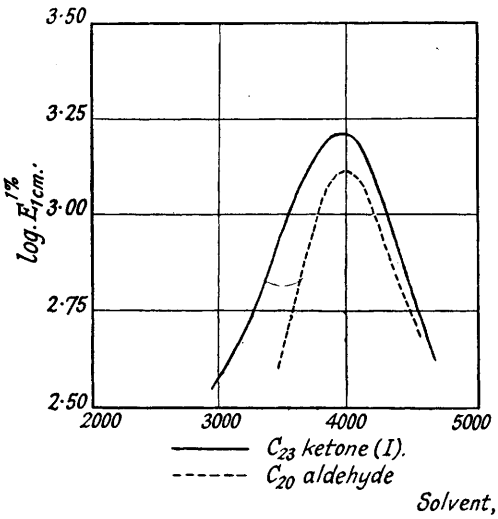
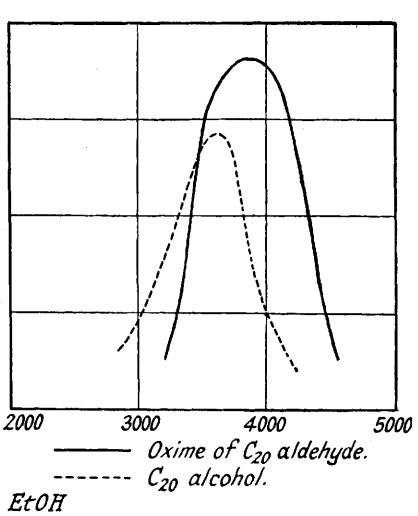


FIG. 3.



Solvent, EtOH

methylsuccinic acid, the isolation of which would definitely establish the presence of the safranal ring. In addition we are investigating the oxidation of β-*cyclo*geraniol under the same conditions with the object of determining whether this reaction is a characteristic of the β-ionone ring.

EXPERIMENTAL.

Oxidation of Vitamin-A.—A vitamin-A concentrate (13 g., $E^{1\%}_{1\,cm.}$ 3280 A. = 1350), aluminium *tert.*-butoxide (15·6 g.), and diethyl ketone (63 g.) were refluxed together in dry A.R. benzene (450 c.c.) in a stream of nitrogen for 48 hours. The resultant suspension was diluted with water, and the benzene layer, after drying over sodium sulphate, evaporated under reduced pressure. The dark red residue was dissolved, together with Girard reagent P (20 g.) in absolute alcohol (260 c.c.) containing glacial acetic acid (25 c.c.) and heated under reflux for 1 hour; the solution was poured into ice-water and, after neutralisation of 90% of the acetic acid with sodium bicarbonate, was repeatedly extracted with ether. It was found that, by shaking the ethereal extract with water and combining the washings with the original aqueous layer, the yield of the aldehyde was materially increased. The total water-soluble fraction was made acid to Congo-red with dilute sulphuric acid and kept at room temperature for 2 hours. Ether extraction of the regenerated aldehyde and removal of the solvent from the washed and dried extract gave a viscous oil (5·3 g.), which was dissolved in light petroleum and adsorbed on a column of calcium hydroxide. Prolonged washing of the chromatogram with light petroleum gave a pale yellow filtrate, from which the aldehyde was obtained as a yellow oil, $E^{1\%}_{1\,cm.}$ 4010 A. = 1430.

Oxime. A solution of the aldehyde (1 g.) in dry pyridine (20 c.c.) was treated with a solu-

tion of free hydroxylamine (1 g.) in the minimum volume of absolute alcohol and warmed at 60° in an atmosphere of nitrogen. After 4 hours the orange solution was diluted with water until cloudy and kept at 0° for several hours. Crystallisation of the precipitate first from light petroleum and finally from absolute alcohol gave the *oxime* as orange rosettes, m. p. 176—177° (Found : C, 80·6, 80·5; H, 9·8, 10·0; N, 4·6, 4·5. $C_{20}H_{27}ON$ requires C, 80·8; H, 9·2; N, 4·7%. Calc. for $C_{20}H_{29}ON$: C, 80·2; H, 9·8; N, 4·7%). These figures may possibly indicate that the oxime (isolated in relatively poor yield) is derived from a small quantity of the authentic vitamin-A aldehyde present in the oxidation product.

Condensation of the Aldehyde with Acetone.—(a) *Sodium ethoxide.* A solution of sodium ethoxide (from 0·5 g. of sodium in 8 c.c. of absolute alcohol) was slowly added with stirring at — 5° to a solution of the aldehyde (1 g.) in pure dry acetone (40 c.c.). After 3 hours' stirring, the alkali was neutralised with a dilute aqueous solution of tartaric acid, and the precipitated oil extracted with ether. After removal of solvent from the washed and dried extract the residue was dissolved in light petroleum and adsorbed on a column of alumina, the lower dark red zone being eluted with light petroleum–methyl alcohol (9 : 1), and the product isolated in the usual manner. This was then dissolved together with *p*-tolylsemicarbazide (1 g.) in alcohol (10 c.c.) containing 2 drops of glacial acetic acid and heated for 10 minutes on the steam-bath. The solid which separated on slight dilution with water and standing overnight, after repeated crystallisation from ethyl acetate, gave the p-*tolylsemicarbazone* as yellow needles, m. p. 206—207° (Found : C, 78·9; H, 8·6; N, 9·0. $C_{31}H_{39}ON_3$ requires C, 79·3; H, 8·4; N, 8·95%). Admixture with the *p*-tolylsemicarbazone of the ketone (I), m. p. 217—218°, depressed the m. p. to 200—203°.

(b) *Aluminium* tert.-*butoxide.* The aldehyde (0·8 g.) was dissolved in dry benzene (100 c.c.) and after addition of acetone (25 c.c.) and aluminium *tert.*-butoxide (4 g.) the solution was refluxed in nitrogen for 16 hours. The product was worked up exactly as described by Batty, Burawoy, Harper, Heilbron, and Jones (*loc. cit.*); the ketone so obtained formed a *p*-tolylsemicarbazone which was identical with that isolated when sodium ethoxide was used as condensing agent.

Reduction of the C_{20} *Aldehyde.*—The aldehyde (0·5 g.) and aluminium *iso*propoxide (0·6 g.) were dissolved in dry *iso*propyl alcohol (15 c.c.), and the solvent slowly distilled, the bulk of the solution being maintained approximately constant by the addition of fresh *iso*propyl alcohol. When the distillate no longer gave a positive test for acetone, the residue was dissolved in light petroleum and washed successively with 4% phosphoric acid, sodium bicarbonate solution, and water. Removal of solvent from the dried solution gave the alcohol as a viscous oil, characterised by an absorption maximum at 3590 A. (Fig. 3) [Found : active hydrogen atom, 0·94 (Zerewitinov)].

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