CHAPTER IV

Part I - Preparation of Retroretinol from Retinol by Concentrated Hydromic Acid.

Part II - Preparation of Retroretinol from Retinol by Ethanolic Hydrogen Chloride.
PART I

Introduction:

Oroshnik et al\(^1\) reported that a synthesis designed to give retinyl methyl ether led to a new series of polyenes, isomeric with the $\alpha$ and $\beta$-ionylidene series. One of these products was found to be retroretinyl methyl ether since it differed from retinyl methyl ether only in that the conjugated pentaene system was displaced one carbon atom into the ring. It had three absorption maxima at 333, 348 and 367 $\text{m} \mu$. Retroretinyl methyl ether thus synthesised was found to be biologically inactive.

Beutel et al\(^2\) prepared retroretinyl acetate from retinyl acetate. Crystalline trans-retinyl acetate in methylene dichloride was mixed with ice-cold concentrated hydrobromic acid by shaking for 30 seconds. The resulting solution after washing with 5 per cent. aqueous sodium bicarbonate solution followed by water was evaporated to dryness under reduced pressure. The residue on chromatography on neutral alumina yielded trans-retroretinyl acetate as a yellow oil. This sample had a value of 56,800 for molecular extinction co-efficient.

The occurrence of retroretinol in multivitamin A preparations was reported by Varma et al\(^3,4\). Commercial samples of multivitamin A preparations were analysed and it was found that most of them contained anhydroretinol, retroreti-
nyl ether, retinyl ether and retroretinol in varying proportions and that the first two were present in greater amounts than the other retinol derivatives. The potency of retroretinyl acetate was found to be 12 per cent. of that of retinol.

The major impurity in samples of synthetic retinyl acetate was identified by thin layer and paper chromatography and ultraviolet absorption spectrum studies as retroretinyl acetate. Varma et al also applied thin layer chromatography to separate retinol, retroretinol and other related compounds.

The conversion of retroretinol to retinol was reported by Varma and Murray and Murray and Erdody. Retinol deficient rats were dosed orally with retroretinol and then sacrificed after the last dose. The livers were extracted and the extract was saponified. Thin layer chromatography of the unsaponifiable material indicated the presence of retinol and retroretinol.

EXPERIMENTAL

Following the procedure outlined by Beutel, Hinkle and Pollak, attempts were made to prepare retroretinyl acetate. Experimental details are given below.
1. Action of concentrated hydrobromic acid on retinyl acetate:
Preparation of retroretinyl acetate:

1.1 Synthetic retinyl acetate (0.25 g) was dissolved in methylene dichloride (3 ml) taken in a 50 ml conical flask. The solution was cooled to 0°C by placing it in a mixture of ice-salt. In another small flask concentrated hydrobromic acid (0.3 ml) was cooled to 0°C by keeping it in the same cooling mixture. The cooled hydrobromic acid was then mixed with the solution of retinyl acetate and the mixture shaken for 30 seconds at 0°C. The reaction mixture was taken out of the bath and placed in a separating funnel. The organic layer was washed several times with 5 per cent. aqueous sodium bicarbonate solution and finally with distilled water and then dried over anhydrous sodium sulphate. The solution was evaporated in vacuo and the yellow oily residue was dissolved in 5-6 ml of light petroleum and chromatographed on a column of neutral alumina.

Preparation of neutral alumina:

100 g of Basic alumina (Merck, for chromatography, standardised according to Brockmann) was put under water in a beaker and dilute hydrochloric acid (0.5N) was added with stirring until just acidic to methyl orange. The alumina was then washed repeatedly with water until it was free from chloride ion, then washed twice with methanol and dried at 140°C for 14 hours and then cooled in a desiccator.
A chromatographic column was packed with neutral alumina weakened by the addition of water (30 g; 5 per cent. of water). The solution containing the reaction product was poured on the column. The chromatogram was developed with light petroleum. A yellow zone was seen moving slowly with light petroleum. The percolate was tested occasionally with 0.5 ml portion of antimony trichloride reagent. When the percolate produced a violet colour with Carr-Price reagent it was then collected in a 250 ml conical flask. The development of the chromatogram was continued as long as the eluate gave violet colour with antimony trichloride reagent. The eluate was concentrated under reduced pressure and the spectral properties were determined.

The eluate exhibited \( \lambda_{\text{max.}} \) at 332, 348 and 367 m\( \mu \) and produced a violet colour with Carr-Price reagent. The antimony trichloride reaction product showed maximum at 575 m\( \mu \) with a minor maximum at 470 m\( \mu \).

1.2 An experiment similar to Expt. No. 1.1 was carried out in which 0.39 g of synthetic retinyl acetate in 4 ml of methylene dichloride was treated with 0.4 ml of concentrated hydrobromic acid at 0° C. The reaction product was taken in light petroleum as before and chromatographed on deactivated neutral alumina (plus 5 per cent. water). The column was washed with light petroleum and the eluate containing the material from the yellow zone was collected. The eluate was studied
with a spectrophotometer. The results are as follows -

\[ \lambda_{\text{max}}. \ 332, 348 \text{ and } 367 \text{ m}\mu \ (\text{in light petroleum}). \]

Antimony trichloride colour \( \lambda_{\text{max}}. 575, 470 \text{ m}\mu \).

1.3 An experiment similar to Expt.No.1.1 was carried out on a larger scale. 0.76 g of synthetic retinyl acetate was dissolved in 8 ml of methylene dichloride and then 0.8ml of concentrated hydrobromic acid was added to this solution at \( 0^\circ \text{C} \). The reaction product was taken in light petroleum as usual and was chromatographed as before. The eluate from the chromatogram after removal of solvent yielded 0.65 g of retroretinyl acetate as yellow oil having \( \lambda_{\text{max}}. \) at 332, 348 and 367 m\mu and antimony trichloride colour \( \lambda_{\text{max}}. \) at 575 m\mu and 470 m\mu.

1.4 Purification of retroretinyl acetate prepared in the above experiments:

Retroretinyl acetate (0.5 g) prepared in the above experiments was dissolved in 10 ml of light petroleum and chromatographed on a column of deactivated neutral alumina (40 g; 5% water). A light yellow zone was formed on the chromatogram. This zone was moving slowly on development with light petroleum. Development was continued with light petroleum. From time to time the percolate was tested with 0.5 ml portion of Carr-Price reagent. The portions that did not give any colour with Carr-Price reagent were rejected. The retro-
**Fig. 25.** Absorption Spectrum of Retroretinyl acetate.

**Fig. 26.** Absorption Spectrum of the SbCl₃ Product of Retroretinyl acetate.
retinyl acetate appeared in the eluate that produced a violet colour with antimony trichloride reagent. At this stage the percolate was collected in small portions (8-10 ml) in test tubes. When the whole of the retroretinyl acetate had passed through the column the development was stopped.

The fractions of the eluate were examined spectrophotometrically and the portions showing ultraviolet absorption maxima at 332, 348 and 367 mμ were combined together. The solvent was removed in vacuo at low temperature. The residue was redissolved in a few ml. of light petroleum. The solution was then transferred quantitatively to a 100 ml. standard flask and the volume was made up. The ultraviolet absorption spectrum of the solution was recorded after appropriate dilution with light petroleum. The amount of retroretinyl acetate present in the solution was determined (Ref. Expt.No.1,2, Chapter III, Part I).

The purified sample of retroretinyl acetate obtained above exhibited λmax. at 332, 348 and 367 mμ. Fig.25, and had values of 1055, 1367 and 1061, respectively, for E(1%,1cm). With antimony trichloride reagent it produced a violet colour and the reaction product showed λmax. at 575mμ with a minor maximum at 470 mμ. Fig.26.

The results of all the experiments are given in Table XVII.
The yield of retroretinyl acetate was calculated from the extinctions at 332, 348 and 367 m\(\mu\), assuming the E(1\%1cm) values at these wavelengths to be 1055, 1367 and 1061, respectively (Ref. Expt. No. 1.4). The results show that maximum yield of retroretinyl acetate is ca. 85.5 per cent.

CHARACTERISATION OF RETRORETINYL ACETATE

2. Hydrolysis of retroretinyl acetate:

Retroretinyl acetate (0.5 g) prepared in Expt. No. 1 above was saponified with a freshly prepared ethanolic KOH solution by heating for 15-20 minutes on a water bath at 50-60\(^\circ\)C. The alcoholic soap solution was cooled and diluted with an equal volume of water and extracted five times with suitable volumes of light petroleum. The extracts were combined
together and washed as usual. The solution was dried over anhydrous sodium sulphate.

The light petroleum extract was concentrated to 8-10 ml under reduced pressure and the solution transferred to a chromatographic column packed with neutral alumina weakened by the addition of 5 per cent. (v/w) of water. A single yellow zone adhered at the top of the column. On development with light petroleum the yellow zone moved slowly down the column and remained fixed at a position about 2 cms from the top of the column. The zone was so strongly adsorbed that it could not be eluted with light petroleum. Therefore, the chromatogram was extruded and the substance eluted with diethylether. The ether was removed and the residue dissolved in a few ml. of light petroleum and the spectral properties were studied.

The substance exhibited \( \lambda_{\text{max.}} \) at 332, 348 and 367 m\( \mu \). It produced a bluish-violet colour with antimony trichloride reagent and the reaction product showed absorption maximum at 580 m\( \mu \).

3. **Flowing Chromatography of retroretinol:**

0.75 g of retroretinyl acetate prepared in Expt. No. 1 was saponified with alcoholic KOH solution as described in Expt. No. 2 and the unsaponifiable matter was extracted with light petroleum as usual. The volume of the solution was reduced to about 10 ml in vacuo and then chromatographed on a column of deactivated neutral alumina (30 g; 5\% water).
Fig. 27. Absorption Spectrum of Retroretinol.

Fig. 28. Absorption Spectrum of the SbCl₃ product of Retroretinol.
Retroretinol formed a yellow zone which was adsorbed strongly and did not move when developed with light petroleum. The column was then developed with light petroleum containing an increasing amount of diethyl ether (1-10%, v/v). The yellow zone containing retroretinol could be eluted with a mixture of ether:light petroleum (10:90). The eluate at this stage was collected in small portions, 8-10 ml, in test tubes.

All the fractions of the eluate were examined spectrophotometrically. Those fractions showing $\lambda_{\text{max.}}$ at 332, 348 and 367 $\mu\text{m}$ were pooled together. The solvent was removed under reduced pressure and the residue redissolved in a few ml. of light petroleum. The light petroleum solution was transferred quantitatively to a 100 ml standard flask and the volume was made up. The ultraviolet absorption spectrum of this was recorded after appropriate dilution. The amount of the substance present in the solution was determined by the procedure described in Expt.No.1.2, Chapter III, Part I.

The purest sample of retroretinol obtained by repeated chromatography exhibited $\lambda_{\text{max.}}$ at 332, 348 and 367 $\mu\text{m}$, ($\text{Fig.27}$), with $E(1\% ,1cm)$ values of 1251, 1579 and 1257, respectively, at the points of maximum absorption in light petroleum. It produced a bluish-violet colour with antimony trichloride reagent showing $\lambda_{\text{max.}}$ at 580 $\mu\text{m}$. ($\text{Fig.28}$).

4. **Infrared spectrum of retroretinol:**

The infrared spectrum of retroretinol, obtained after saponification of its acetate prepared in Expt.No.1,
Fig. 29. Infrared Spectrum of Retroretinol.
was examined and the following bands (in cm\(^{-1}\)) were observed. (Fig. 29).

| 3400 (s) | 1470 (s+N) | 1045 (m) |
| 2960 (s+N) | 1382 (s+N) | 960 (s) |
| 2850 (s+N) | 1205 (ms) | 880 (ms) |
| 1750 (w) | 1125 (m) | 825 (w) |
| 1680 (w) | 1085 (m) | 802 (w) |
| | | 725 (w) |

s=sharp; w=weak; m=medium; N=due to nujol

5. Oxidation of retroretinol with manganese-dioxide:

5.1 Preparation of retinal:

A wad of cotton was introduced into the constriction of a glass chromatographic column (dia. 1.1 cm) and manganese-dioxide (0.500 g, B.D.H., precipitated) was packed into it. A solution of retroretinol (0.020 g) in light petroleum (5 ml) was poured on to the column. When the whole of the solution had passed, the column was washed with light petroleum (10 ml). The combined percolate which had a light yellow colour was evaporated to dryness in vacuum and the residue dissolved in light petroleum (5 ml) and chromatographed on de-activated alumina (20 g; 8 per cent. water). A yellow zone was eluted with petroleum ether containing 2 per cent. (v/v) of ether. The ultraviolet absorption spectrum of the material in light petroleum was recorded.
The spectrum showed no significant selective absorption and hence the substance might be a decomposition product. The proportion of manganese-dioxide was similar to that recommended by Ball et al for the oxidation of retinol. For retroretinol, therefore, this proportion might have been too high. Hence in the subsequent experiments the proportion of MnO₂ was reduced.

5.2 A column of manganese-dioxide (0.4 g, B.D.H., precipitated) was prepared as in the above experiment and a solution of retroretinol (0.020 g) in light petroleum (5 ml) was percolated through it. The percolate showed a deeper, orange yellow colour compared to the colour of the original solution. The column was washed with light petroleum (10 ml) and ether-petroleum ether mixture (5 ml; 10% ether). All the percolates were combined together. The combined percolate was evaporated to dryness in vacuo and the residue was dissolved in light petroleum (5 ml) and finally chromatographed on deactivated alumina (20 g; 8% water). Development with light petroleum resulted in the separation of two distinct zones: the lower one was an orange zone and above this was a yellow zone. Development with a mixture of 2 per cent. (v/v) of ether in light petroleum effected a quick separation of the zones and the lower zone finally flowed out of the column. The other zone was eluted after extrusion, with diethyl ether and after removal of the ether in vacuo the substance was taken in light petroleum. The ultraviolet absorption spectra of the fractions were determined.
Fig. 30. Absorption Spectrum of Retinal in light petroleum.

Fig. 31. Absorption Spectrum of the SbCl₃ product of Retinal.
The eluate obtained with 2% ether exhibited $\lambda_{\text{max}}$ at 369 $\mu\text{m}$ in light petroleum, (Fig. 3C) (380 $\mu\text{m}$ in ethanol) and produced a green colour with antimony trichloride reagent. The antimony trichloride reaction product showed $\lambda_{\text{max}}$ at 660 $\mu\text{m}$ (Fig. 31). Thus this fraction, from the orange colour of its solution, its ultraviolet absorption maximum and antimony trichloride colour maximum appeared to be retinal.

The second yellow zone which was eluted with ether after extrusion, showed $\lambda_{\text{max}}$ at 332, 348 and 367 $\mu\text{m}$ in light petroleum and gave a bluish-violet colour with Carr-Price reagent. The antimony trichloride colour absorption maximum was at 580 $\mu\text{m}$. All these showed that this fraction contained unreacted retroretinol.

Based on an $E(1\%, 1\text{cm})$ value of 1685 for pure retinal at 369 $\mu\text{m}$ in light petroleum, the amount of retinal obtained was calculated at 0.00103 g and this represented a 5.1% conversion of retroretinol to retinal.

5.3 Expt. No. 5.2 was repeated under similar conditions but varying the proportion of manganese-dioxide. The results are shown in Table XVIII. (p. 166).

The yield of retinal formed was calculated assuming $E(1\%, 1\text{cm})$ value of 1685 for pure retinal at 369 $\mu\text{m}$ in light petroleum. A maximum of 6% conversion of retroretinol to retinal was achieved when a 15 fold proportion of manganese-dioxide was used.
Table XVIII
Oxidation of retinol to retinal.
Temperature = 30°C.

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Wt. of retroretinol (mg)</th>
<th>Wt. of manganese-dioxide (mg)</th>
<th>Wt. of retinal (mg)</th>
<th>Conversion to retinal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.2</td>
<td>20</td>
<td>400 (20 fold)</td>
<td>1.03</td>
<td>5.1</td>
</tr>
<tr>
<td>5.3</td>
<td>20</td>
<td>360 (18 fold)</td>
<td>1.1</td>
<td>5.5</td>
</tr>
<tr>
<td>5.4</td>
<td>20</td>
<td>300 (15 fold)</td>
<td>1.2</td>
<td>6.0</td>
</tr>
<tr>
<td>5.5</td>
<td>20</td>
<td>200 (10 fold)</td>
<td>0.7</td>
<td>3.5</td>
</tr>
<tr>
<td>5.6</td>
<td>20</td>
<td>440 (22 fold)</td>
<td>0.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

6. Reduction of retinal with sodium borohydride:

Preparation of retinol:

Retinal (10 mg; obtained in Expt. No. 6) was dissolved in ethanol (10 ml). The solution was cooled in an ice-bath and 5 mg. of sodium borohydride was added. The mixture was shaken for sometime in the ice-bath when the solution turned almost colourless. The reaction mixture was taken out from the ice-bath. Water was added and the product was extracted with ether. The ethereal extract was washed with water and subsequently dried over anhydrous sodium sulphate. The residue obtained after removal of the ether by evaporation under reduced pressure was dissolved in light petroleum.

The product was chromatographed on a column of dea-
civated alumina (plus 5% water). A single pale yellow zone
Fig. 32. Absorption Spectrum of Retinol.
was found to be formed in the upper region of the column. The chromatogram was developed first with light petroleum and then with a mixture of diethyl ether and light petroleum containing gradually increasing amounts of diethyl ether (1-10%; v/v). The light yellow zone, however, could not be eluted. The chromatogram was extruded carefully and the material from the yellow zone was eluted with ether. The ether was then removed and the residue was dissolved in light petroleum. The spectral characteristics of the substance were examined. The following results were obtained.

\[ \lambda_{\text{max.}} \ 325 \text{ m}\mu \ (\text{in light petroleum}). \text{Fig.32.} \]

Antimony trichloride blue colour maximum - 615-617 m\mu. Fig.33.

The experiment was repeated several times and retinol was obtained in all the experiments.

7. Anhydroretinol from retinol:

50 mg. of Retinal, obtained from Expt.No.6, was reduced with sodium borohydride in ethanol was described in the preceding experiment. The retinol formed (\[ \lambda_{\text{max.}} \ 325 \text{ m}\mu; \ SbCl_3 \text{ reaction product, } \lambda_{\text{max.}} \ 617 \text{ m}\mu \]) was separated by chromatography as before and was taken in light petroleum.

The light petroleum solution containing retinol was evaporated to dryness under reduced pressure and the residue was treated with 0.033N ethanolic hydrogen chloride (25 ml). The reaction mixture was kept at room temperature for 15
Fig. 33. Absorption Spectra of the SbCl₃ products of 1. Retinol, 2. Anhydroretinol.

Fig. 34. Absorption Spectrum of Anhydroretinol.
minutes in the dark. The resulting greenish-yellow solution was then neutralised with a suitable amount of sodium bicarbonate and the product extracted with light petroleum. The extract was washed several times with distilled water, dried over anhydrous sodium sulphate and concentrated to 5-6 ml under reduced pressure. The solution was next chromatographed with 10 g of deactivated alumina (5% water). On development with light petroleum a yellow zone passed quickly through the column. The solution percolating out was collected and examined spectrophotometrically.

The solution showed absorption triplet characteristic of anhydroretinol with \( \lambda_{\text{max}} \) at 350, 369 and 390 \( \mu \)m with an inflection at 335 \( \mu \)m (Fig. 34). It produced a blue colour with Carr-Price reagent and the reaction product exhibited \( \lambda_{\text{max}} \) at 617 \( \mu \)m (Fig. 33).

Repetition of the experiment showed the results to be reproducible.

8. Chromatography of a mixture of retinal from retroretinol and that from retinol:

8.1 Retinol (0.050 g), obtained by saponification of synthetic retinyl acetate, was dissolved in 10 ml of light petroleum. Manganese-dioxide (1.2 g, B.D.H. precipitated) was taken in a chromatographic column and the light petroleum solution of retinol was poured in gently at the top of the column. The percolate was collected and the column was washed.
with light petroleum (20 ml). A deep yellow coloured solution was obtained. Washing of the column was continued until the washed liquid gave no greenish colour with Carr-Price reagent. The percolate and the washings were pooled together and concentrated under reduced pressure to about 10 ml.

The solution was next chromatographed on 10 g of deactivated alumina (8% water). On development with light petroleum an orange yellow zone was seen to be moving slowly. This zone could be eluted with light petroleum and the eluate was transferred to a 100 ml standard flask after concentration under reduced pressure. The ultraviolet spectrum of the solution was recorded after appropriate dilution. This substance produced a greenish colour with Carr-Price reagent. The absorption maximum of the antimony trichloride reaction product was determined as follows:

\[ \lambda_{max} = 369 \text{ m}\mu \text{ (in light petroleum)} \]
\[ 380 \text{ m}\mu \text{ (in ethanol).} \]

Antimony trichloride product: \[ \lambda_{max} = 660 \text{ m}\mu. \]

8.2 A solution of retinal (about 5 mg) in light petroleum, obtained from retroretinol in Expt. No. 5, was mixed with about 5 mg of retinal obtained from retinol (vide Expt. No. 9.1) and the mixture was chromatographed on 20 g of deactivated alumina (plus 8% water). The column was washed with light petroleum and then with light petroleum containing 2% (v/v) of ether. No zone separation was seen when the column
was examined under the ultraviolet light. A single orange yellow zone was finally eluted with light petroleum containing 2 per cent. (v/v) of ether.

The eluate was evaporated to dryness and the residue dissolved in light petroleum. The spectral characteristics of the substance were examined. The following results were obtained.

\[ \lambda_{\text{max.}} = 369 \text{ m}\mu \text{ (in light petroleum)} \]
\[ = 380 \text{ m}\mu \text{ (in ethanol).} \]

Antimony trichloride product: \[ \lambda_{\text{max.}} = 660 \text{ m}\mu. \]

This and the preceding two experiments, viz., Expt. Nos. 6 and 7, proved the identity of retinal from retroretinol and retinal from retinol.

9. Action of ethanolic hydrogen chloride on retroretinol:

Retroretinol obtained by the saponification of its acetate was treated with ethanolic hydrogen chloride to examine whether it could be dehydrated like retinol.

10 mg. of retroretinol was dissolved in 20 ml of 0.033N ethanolic hydrogen chloride in the dark at room temperature and allowed to stand for 15 minutes. The reaction mixture was neutralised with an aqueous solution of sodium bicarbonate and the product extracted with light petroleum. The extract was washed and dried as usual. The volume of the solution was reduced to 3-4 ml. under reduced pressure and chromatographed on a column of weakened alumina (5% water). A
single yellow zone was observed to form at about 2 cms from the top of the chromatographic column. The chromatogram was developed with light petroleum and the eluate was examined with the help of a spectrophotometer. The eluate showed no characteristic absorption in the range of 270 m\textmu to 400 m\textmu of the spectrum.

The yellow zone was finally eluted with light petroleum containing 10 per cent.(v/v) of ether. The solvent was removed under reduced pressure and the residue in light petroleum proved to be retroretinol, $\lambda_{\text{max}}$ 332, 348 and 367 m\textmu; antimony trichloride colour absorption maximum at 580 m\textmu.

It appeared from this experiment that unlike retinol, retroretinol could not be dehydrated with ethanolic hydrogen chloride.

10. Behaviour of Retroretinyl acetate and retroretinol on alumina column during chromatography:

10.1 On basic alumina:

Retroretinyl acetate (20 mg) was dissolved in light petroleum (5 ml) and subjected to chromatography on deactivated alumina (10 g, 5\% water). A yellow zone was found to be formed which was moving slowly on development with light petroleum. The material from this zone was finally eluted with light petroleum. The eluate showed $\lambda_{\text{max}}$ at 332, 348 and 367 m\textmu and antimony trichloride colour absorption maximum at 575 m\textmu with a minor maximum at 470 m\textmu.
On the other hand when chromatographed under similar condition retinol was adsorbed more strongly by alumina and could be eluted with a mixture of diethyl ether:light petroleum (10:90).

10.2 On neutral alumina:

An experiment similar to Expt. No. 10.1 was carried out in which retroretinyl acetate (20 mg) was chromatographed on a column of neutral alumina (prepared as in Expt. No. 1) weakened by the addition of 5 per cent. of water. Retroretinyl acetate was found to form a yellow zone which could be eluted with light petroleum as before.

Retroretinol showed a similar behaviour on a column of neutral alumina as it did with basic alumina.

**DISCUSSION**

The applicability of the method as outlined by Beutel et al. for the conversion of retinyl acetate to retroretinyl acetate was tried successfully in the present work. The method is very simple and the conversion can be effected in less than a minute. An 85.5 per cent. yield was recorded.

On chromatography the reaction product formed a bright yellow zone on a column of neutral alumina deactivated by the addition of 5 per cent. of water. This yellow zone containing retroretinyl acetate was moving slowly when
developed with light petroleum and finally flowed out of the column. The extent of conversion was quite satisfactory and no retinyl acetate was left in the reaction mixture. Only a very thin yellow zone probably containing the decomposition product adhered at the top of the column.

Retroretinyl acetate thus prepared was purified by flowing chromatographic method using water-deactivated neutral alumina. Fractions in light petroleum were collected and the ultraviolet absorption spectrum of each of them was recorded. The fractions showing \( \lambda_{\text{max.}} \) at 332, 348 and 367\( \mu \) were pooled together. The solvent was removed under reduced pressure and the values of \( E(1\% \text{, } 1 \text{cm}) \) of the residue at the points of maximum absorption were determined. The purest sample of retroretinyl acetate exhibited \( \lambda_{\text{max.}} \) at 332, 348 and 367 \( \mu \) and had values of 1055, 1367 and 1061, respectively, for \( E(1\% \text{, } 1 \text{cm}) \).

The characterisation of retroretinyl acetate prepared here was made from the following observations: (1) the ultraviolet absorption maxima of this compound tally with those of retroretinyl acetate obtained by Beutel et al\(^2\) and other workers\(^5\). The antimony trichloride colour absorption maximum was found to be at 575 \( \mu \). (2) On saponification with alcoholic KOH the compound yielded an alcohol whose spectroscopic properties are identical with those of retroretinol. (3) Retroretinol obtained from its acetate showed in the infrared spectrum characteristic band for \(-\text{OH}\) group.
Retroretinyl acetate was found to produce a violet colour with antimony trichloride reagent. The reaction product exhibited $\lambda_{\text{max.}}$ at 575 m$\mu$ with a subsidiary peak at 470 m$\mu$. But retroretinol (obtained from retroretinyl acetate on saponification) produced a bluish-violet colour with antimony trichloride reagent showing $\lambda_{\text{max.}}$ at 580 m$\mu$.

Retroretinol obtained by the hydrolysis of its ester was found to be resistant to ethanolic hydrogen chloride. The product recovered from the reaction mixture was found to exhibit all the characteristic properties of retroretinol (vide Expt. No. 9).

Retroretinyl acetate was found to behave identically on the chromatographic column prepared with both basic and neutral alumina. Similarly, retroretinol also behaved identically.

Like retinol, retroretinol yielded retinal on oxidation with manganese-dioxide. But the yield and the ratio of retroretinol to manganese-dioxide used are different from those of retinol. The yield of retinal from retroretinol was maximum, viz. ca. 6% when 15-fold manganese-dioxide was used to oxidise retroretinol. If this proportion is increased the probability of retinal formation becomes less due to the formation of some other compound having no characteristic absorption. Decrease in the amount of manganese-dioxide was found to result in decrease of the yield of retinal.
Retinal thus obtained was characterised spectrophotometrically: $\lambda_{\text{max}}$ 369 $\mu$m in light petroleum and 380 $\mu$m in ethanol. With antimony trichloride it produced a green colour showing maximum at 660 $\mu$m. The characterisation of retinal was further made by reducing retinal in ethanol with sodium borohydride when retinol was obtained, $\lambda_{\text{max}}$ 325 $\mu$m; SbCl$_3$ reaction product $\lambda_{\text{max}}$ at 615-617 $\mu$m. Retinol thus obtained was converted into anhydroretinol having absorption triplet at 350, 369 and 390 $\mu$m (in light petroleum) by the action of ethanolic hydrogen chloride. The identity of retinal was further established by chromatographing a mixture of retinals, - one prepared from retroretinol and the other from retinol.

**The mechanism of formation of retinal:**

Wald$^{10}$ recognised that the mechanism involved in Morton’s procedure for the oxidation of retinol was a preliminary adsorption of the alcohol on the manganese dioxide and its subsequent oxidation in the adsorbed state itself to retinal. This enabled him to effect "chromatographic oxidation" of retinol on a column of manganese dioxide. On the column, retinol is first adsorbed and oxidised to retinal. Retinal itself is much less strongly adsorbed than retinol and as soon as it is formed, unreacted retinol displaces it. More of retinol thus comes into contact with the adsorbent and the oxidation continues. According to Wald "processes
like this where a solid acts at once as adsorbent and reagent may mimic the specificity and directedness of enzymic reactions. Meunier et al. have, for example, claimed to have obtained a 50% yield of retinal by oxidation of β-carotene, on manganese dioxide.

The quality of manganese dioxide used was found to exercise profound influence on the course of the oxidation. The manganese dioxide that was capable of oxidising the allylic methylene group in retinol and retinal to a keto group was one prepared under acidic conditions. Attenburrow et al. who oxidised several primary and secondary alcohols to the corresponding carbonyl compounds, on the other hand, found that the most active samples were those precipitated under alkaline conditions. Even the final drying of the preparation was critical as both overdrying and underdrying could greatly reduce the activity of the oxide. The active material was a hydrated oxide.

In the present investigation, it is found that oxidation of retroretinol(I) on a manganese dioxide column produces a small yield of retinal(II). The formation of retinal from retroretinol whose structure differed from that of retinol in that the conjugated pentaene system is displaced one carbon atom into the ring, would therefore mean that oxidation has taken place at the end primary alcoholic group. A shift of the "retro" system of conjugation in retroretinol to the normal structure of retinol has taken place in the
This is an unusual reaction and to suggest a mechanism for it is far from simple. The first step may probably be the strong adsorption of retroretinol on the manganese dioxide. During the process of adsorption, the hydroxyl group attaches itself firmly to the adsorbent surface and in doing so splits itself from the body of the molecule giving a carbonium ion with a positive charge at C$_{15}$(III). The pentaene system in this ion then undergoes an allylic rearrangement producing carbonium ion(IV) which is oxidised to retinal (Ref. oxidation of 3-hydroxy anhydroretinol to 3-dehydroretinol$^1$).
The ionisation of retroretinol visualised above is similar to that of retinol proposed by Meunier\textsuperscript{14} in seeking to explain the colours formed on adsorption of retinol on acid clays. In fact, some varieties of manganese dioxide have been actually shown to act as ion exchangers like zeolites\textsuperscript{15}.

An allylic rearrangement of the type envisaged cannot, probably, take place with facility in a heterogeneous reaction, although in homogeneous reactions such as the acid-catalysed 'dehydration' of retinol it is extremely easy. Also, there is a reversion of the stabler(?) retro-structure\textsuperscript{1} of retroretinol to the normal structure of retinol. It is probably these reasons that restrict the yield of retinal.

The mechanism of heterogeneous reactions in general is not well understood\textsuperscript{16} and the various types of manganese dioxide oxidation prove no exception. Attenburrow et al\textsuperscript{12} found that the active variety of manganese dioxide prepared by them was hydrated and it is believed\textsuperscript{17} that the presence of this bound water is greatly responsible for the specific activity of the oxide.
PART II

Introduction:

Although Beutel et al \(^2\) reported the preparation of retroretinyl acetate from retinyl acetate by the action of concentrated hydrobromic acid, no information is available in literature for the preparation of retroretinol by the action of hydrogen chloride on retinol. However, Barua and Rao \(^9\) reported the separation of a compound, whose absorption spectrum exhibited \(\lambda_{\text{max}}\) at 330, 350 and 370 m\(\mu\), by chromatographing the reaction mixture obtained by the action of ethanolic hydrogen chloride on retinol. This substance was suspected by them to be rehydrorotenol(?).

In the present investigation attempts have been made to examine whether retinol can be converted to retro-retinol by the treatment with dry ethanolic hydrogen chloride.

EXPERIMENTAL

1. Action of ethanolic hydrogen chloride on retinol:
   (Ref.Expt.Nos. 1 & 2,Ch.III, Part II).

   Preparation of retroretinol:

1.1 A known weight of retinol was treated with 0.033N ethanolic hydrogen chloride (100 ml) for 40 minutes at room temperature in the dark. The reaction product was extracted with light petroleum as usual. The light petroleum extract was chromatographed on a column of deactivated alumina.
(5% water) when anhydroretinol quickly passed through leaving a yellow zone at the top of the column. Development was continued with light petroleum when the single yellow zone separated into three different ones. The description of these three yellow zones and the spectral characteristics of each of them were given in Expt. No. 1.2, Chapter III, Part II.

The substance showing $\lambda_{\text{max.}}$ at 290 $\mu\text{m}$ and eluted from the yellow zone occupying the top position in the chromatogram, was proved to be kitol (Ref. Chapter III, Part II).

The material from the second yellow zone exhibiting $\lambda_{\text{max.}}$ at 332, 348 and 357 $\mu\text{m}$ was taken up for characterisation in the present study.

1.2 Purification of the substance from the second yellow zone, obtained in Expt. No. 1.1, by repeated chromatography:

The material from the second yellow zone was dissolved in a few ml. of light petroleum and was transferred to a chromatographic column packed with deactivated alumina (5% water). The chromatogram was developed first with light petroleum and then with a mixture of diethyl ether and light petroleum containing gradually increasing amount of diethyl ether. The mixture finally used contained 10 per cent. (v/v) of ether. The percolate at this stage was collected in small portions (8-10 ml) in test tubes. One drop from each portion was tested with 0.5 ml of Carr-Price reagent. The first few portions of the percolate gave no colour and were discarded.
Fig. 25. Absorption Spectrum of Retroretinol.
Continued development with light petroleum containing 10% ether resulted in the appearance of a bluish-violet colour with Carr-Price reagent. After a volume of 200-250 ml had passed through the column no colour appeared with the Carr-Price reagent and then the development was discontinued.

The ultraviolet absorption spectra of all the portions were recorded and the portions exhibiting \( \lambda_{\text{max}} \) at 332, 348 and 367 m\( \mu \) were pooled together. The solvent was removed under reduced pressure and the residue dissolved in a few ml. of light petroleum.

1.3 Extent of conversion of retinol to retroretinol:

The light petroleum solution containing the purified substance from the yellow zone was transferred to a 100 ml standard flask and the volume was made up with light petroleum. The ultraviolet absorption spectrum of this solution was recorded in the region 270-400 m\( \mu \) after appropriate dilution. The amount of the purified substance present in the solution was determined following the procedure as described in Expt.No.1.2, Chapter III, Part I. The extent of conversion of retinol to retroretinol was then calculated assuming that in a 100 per cent. conversion 286 g of retinol yielded 285 g of retroretinol.

The purified sample of the compound thus obtained \( \lambda_{\text{max}} \) at 332, 348 and 367 m\( \mu \) (Fig.35) and had values of 1270, 1593 and 1274, respectively for \( E(1%,1\text{cm}) \). With Carr-
Fig. 36. Absorption Spectrum of the SbCl$_3$ product of Retinol.
Price reagent it produced a bluish-violet colour and the reaction product showed \( \lambda_{\text{max.}} \) at 580 \( \text{m} \mu \). Fig.36.

These spectral characteristics are attributed to the compound retroretinol\(^2\). Hence, it is concluded that the compound in the yellow zone of the chromatogram is nothing but retroretinol.

Experiment No.1.1 was repeated several times, keeping the amount of retinol the same (150 mg) in 100 ml of ethanolic hydrogen chloride (0.033N). The yield of retroretinol was calculated from the extinction at 348 m\( \mu \) assuming the \( E(1\% , 1\text{cm}) \) value at this wavelength for pure retroretinol to be 1593 (Ref.Expt.No.1.2). Repeated experimentation showed that the conversion of retinol to retroretinol is very poor by this method, being only ca.1.8-2.0 per cent.

The influence of various factors such as concentration of the acid, the duration of reaction and the temperature on the yield of retroretinol from retinol was then investigated (Ref.Expt.Nos. 2, 3 & 4, Chapter III, Part II).

2. Effect of concentration of hydrogen chloride on the formation of retroretinol (Expt.No.2,Ch.III, Part II):

A set of experiments was performed in which retinol (150mg) was treated with ethanolic hydrogen chloride (100 ml) of varying concentrations, viz., 0.033, 0.06, 0.1, 0.13, 0.15 and 0.2N, respectively, at room temperature in the dark. The reaction mixtures were examined quantitatively for retro-
retinol. The results of this set of experiment are given in Table XIX.

Table XIX
Effect of concentration of hydrogen chloride on the formation of retroretinol.

Temperature=30°C.

<table>
<thead>
<tr>
<th>Conc. of the acid (N)</th>
<th>Retinol taken (mg)</th>
<th>Retroretinol formed (mg)</th>
<th>Conversion to retroretinol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.033</td>
<td>150</td>
<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
<td>0.6</td>
<td>150</td>
<td>5.1</td>
<td>3.4</td>
</tr>
<tr>
<td>0.1</td>
<td>150</td>
<td>7.5</td>
<td>5.0</td>
</tr>
<tr>
<td>0.13</td>
<td>150</td>
<td>8.4</td>
<td>5.6</td>
</tr>
<tr>
<td>0.15</td>
<td>150</td>
<td>7.6</td>
<td>5.1</td>
</tr>
<tr>
<td>0.2</td>
<td>150</td>
<td>5.1</td>
<td>4.0</td>
</tr>
</tbody>
</table>

The results show that the yield of retroretinol is maximum, viz. ca 5.5 per cent. at the acid concentration of 0.13N. Therefore, in the subsequent experiments 0.13N ethanolic hydrogen chloride was used for the preparation of retroretinol from retinol.

3. Effect of duration of reaction on the formation of retroretinol (Ref. Expt. No. 3, Chapter III, Part II):

In this set of experiments retinol was treated with 0.13N ethanolic hydrogen chloride (100 ml) at room temperature in the dark. But the duration of the reaction was increased gradually from 40 minutes to 3 hours. The reaction
mixtures were analysed quantitatively for retroretinol. The results are shown in Table XX.

Table XX
Effect of duration of reaction on the formation of retroretinol.
Temperature=31-32°C.

<table>
<thead>
<tr>
<th>Duration of reaction (hour)</th>
<th>Retinol taken (mg)</th>
<th>Retforetinol formed (mg)</th>
<th>Conversion to retroretinol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/4</td>
<td>150</td>
<td>8.4</td>
<td>5.6</td>
</tr>
<tr>
<td>1</td>
<td>150</td>
<td>12.5</td>
<td>8.3</td>
</tr>
<tr>
<td>1 1/2</td>
<td>150</td>
<td>15.0</td>
<td>10.0</td>
</tr>
<tr>
<td>2</td>
<td>150</td>
<td>12.1</td>
<td>8.0</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>8.3</td>
<td>5.5</td>
</tr>
</tbody>
</table>

From the above table it is clear that the conversion of retinol to retroretinol is maximum, in ca 10.0 per cent. when retinol is treated with 0.13N ethanolic hydrogen chloride for 1 1/2 hours at room temperature in the dark. In the subsequent experiments, therefore, the duration of reaction was maintained at 1 1/2 hours.

4. Effect of variation of temperature on the formation of retroretinol (Ref.Expt.No.4, Chapter III, Part II):

A set of experiments was carried out in which retinol was treated with 0.13N ethanolic hydrogen chloride for 1 1/2 hours at different temperatures and the corresponding
Fig. 37. Infrared Spectrum of Retroretinol.
yields of retroretinol were determined. An incubator was used for the variation of temperature. Table XXI shows the yield of retroretinol at various temperatures.

Table XXI

Effect of temperature on the formation of retroretinol.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Retinol taken (mg)</th>
<th>Retroretinol formed (mg)</th>
<th>Conversion to retroretinol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>150</td>
<td>9.9</td>
<td>6.6</td>
</tr>
<tr>
<td>25</td>
<td>150</td>
<td>12.6</td>
<td>8.4</td>
</tr>
<tr>
<td>30</td>
<td>150</td>
<td>15.1</td>
<td>10.1</td>
</tr>
<tr>
<td>35</td>
<td>150</td>
<td>14.4</td>
<td>9.6</td>
</tr>
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<td>40</td>
<td>150</td>
<td>10.6</td>
<td>7.0</td>
</tr>
<tr>
<td>50</td>
<td>150</td>
<td>7.4</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Table XXI shows that the yield of retroretinol from retinol is maximum, viz., ca 10-10.1% between the temperatures 30-32°C.

CHARACTERISATION OF RETRORETINOL

5. Infrared spectrum of retroretinol:

The infrared spectrum of a pure sample of retroretinol (Ecm at 348 μ 1593) was recorded. The following bands (in cm⁻¹) were observed. (Fig.37).
6. Antimony trichloride reaction product of retroretinol:

Retroretinol obtained from retinol in Expt. Nos. 1-4 produced a bluish-violet colour with Carr-Price reagent. A spectrum of the reaction product in the region 450-700 m\(\mu\) was recorded within 20 seconds and a maximum absorption was obtained at 580 m\(\mu\). Fig. 36.

7. Retroretinyl acetate from retroretinol:

7.1 Retroretinol (obtained from retinol in Expt. Nos. 1-4) (15 mg) was dissolved in a mixture containing 1 ml of freshly distilled acetic anhydride and 3 ml of pyridine and the reaction mixture was heated on a water-bath at about 50°C for 30 minutes. The reaction mixture was cooled and then 50 ml of light petroleum was added. The mixture was poured into a solution of 3N hydrochloric acid (100 ml) taken in a separating funnel and mixed thoroughly. The aqueous layer was removed and the organic layer washed with another 100 ml portion of the acid. It was then successively washed with water,
Fig. 38. Absorption Spectrum of the SbCl₃ product of Retroretinyl acetate.

Fig. 39. Absorption Spectrum of the SbCl₃ product of Retroretinyl acetate.
5 per cent. aqueous solution of sodium bicarbonate and water and finally dried over anhydrous sodium sulphate.

The light petroleum extract was concentrated to about 5-6 ml under reduced pressure and then transferred to a chromatographic column packed with alumina weakened by the addition of 5 per cent. of water. The substance formed a yellow zone which separated into two on continued development with light petroleum. These were -

**Zone I.** A yellow zone adsorbed strongly by alumina. The substance from this zone was eluted after extrusion, with diethyl ether. The ether was removed and the residue dissolved in light petroleum. The ultraviolet absorption spectrum showed \( \lambda_{\text{max}} \) at 332, 348 and 367 m\( \mu \). It produced a bluish-violet colour with antimony trichloride reagent: \( \lambda_{\text{max}} \approx 580 \) m\( \mu \).

**Zone II.** A yellow zone moving slowly with light petroleum and finally flowed out. This substance exhibited \( \lambda_{\text{max}} \) at 332, 348 and 367 m\( \mu \). Fig. 38, and produced a violet colour with antimony trichloride reagent showing \( \lambda_{\text{max}} \) at 575 m\( \mu \) with a minor maximum at 470 m\( \mu \)(Fig. 39).

Thus, Zone I was unesterified retroretinol and Zone II contained retroretinyl acetate.

7.2 An experiment similar to Expt. No. 7.1 was carried out in which 50 mg of retroretinol was esterified with 4 ml of a mixture of acetic anhydride and pyridine (1:3). The reaction product was extracted with light petroleum as described in Expt. No. 7.1.
Chromatography of the reaction product in light petroleum on water-deactivated alumina (5% water) resulted in the separation of two yellow zones. One of them was moving slowly on development with light petroleum and finally flowed out. The eluate was concentrated by evaporation under reduced pressure and the spectral properties were determined. The following results were obtained.

\[ \lambda_{\text{max.}} 332, 348 \text{ and } 367 \text{ m\textmu} \text{ in light petroleum.} \]

Antimony trichloride product, \[ \lambda_{\text{max.}} 575 \text{ m\textmu}, \sim 470 \text{ m\textmu}. \]

The other yellow zone sticking near the top of the column was found to contain unconverted retroretinol: \[ \lambda_{\text{max.}} \text{at } 332, 348 \text{ and } 367 \text{ m\textmu}; \text{ antimony trichloride band at } 580 \text{ m\textmu}. \]

Repetition of this experiment showed the results to be reproducible.

Retroretinyl acetate thus prepared was purified by flowing chromatography and the ultimate sample had values of 1069, 1382 and 1073, respectively, for \( E(1\%, 1\text{cm}) \).

8. Hydrolysis of retroretinyl acetate:

Retroretinyl acetate (20 mg), obtained from retroretinol in Expt. No. 7, was saponified with a solution of KOH in ethanol by heating 15-20 minutes on a water bath at 50-60°C. The unsaponifiable matter was extracted with light petroleum as described in Expt. No. 2, Part I of this Chapter. The light petroleum extract was next chromatographed on a column of deactivated alumina (5% water). A single yellow
zone adhered strongly about 2 cms below the top of the column. The chromatogram was extruded and the substance from the yellow zone was eluted with diethyl ether. The ether was removed under reduced pressure and the residue was dissolved in light petroleum.

The light petroleum solution exhibited $\lambda_{\text{max.}}$ at 332, 348 and 357 $\mu\nu$ and produced a bluish-violet colour with antimony trichloride reagent: $\lambda_{\text{max.}}$ 580 $\mu\nu$.

9. Oxidation of retroretinol with manganese dioxide:

(Expt. No. 5, Part I)

Preparation of retinol

Retroretinol (20 mg) prepared from retinol in Expt. Nos. 1-4, was dissolved in 5 ml of light petroleum. The solution was then passed through a column of manganese dioxide (200 mg, B.D.H. precipitated) taken in a chromatographic tube. The percolate which was orange-yellow in colour was collected and the column was washed with light petroleum (10 ml). The percolate and the washing were combined together and concentrated under reduced pressure to about 5-6 ml.

The solution was next chromatographed on a column of weakened alumina (20 g: 8% water). Development with light petroleum resulted in the separation of two zones. One of them, orange-yellow in colour, was moving slowly and could be eluted with light petroleum containing 2 per cent. (v/v) of ether. The solvent was removed under reduced pressure and the residue was dissolved in light petroleum. This
Fig. 40. Absorption Spectrum of Retinal in light petroleum.

Fig. 41. Absorption Spectrum of the SbCl₃ product of Retinal.
fraction exhibited $\lambda_{\text{max}}$ at 369 $\mu$m (Fig. 40) in light petrol­
eum (380 $\mu$m in ethanol) and produced a green colour with antimony trichloride reagent. The reaction product showed $\lambda_{\text{max}}$ at 660 $\mu$m (Fig. 41). Thus, this fraction, from the orange colour of its solution, its ultraviolet absorption maximum and antimony trichloride colour absorption maximum, appeared to be retinal.

The second yellow zone which was strongly adsorbed by alumina, was found to contain unchanged retroretinol: $\lambda_{\text{max}}$ 332, 348 and 367 $\mu$m; antimony trichloride reaction product, $\lambda_{\text{max}}$ 580 $\mu$m.

Based on an $E(\lambda, 1\text{cm})$ value of 1685 for pure retinal in light petroleum at 369 $\mu$m, the amount of retinal obtained was calculated.

Experiment No. 9 was repeated several times under similar conditions. The conversion of retroretinol to retinal was found to take place to the extent of 5.5-6%.

10. Reduction of retinal to retinol with sodium borohydride:

Retinal (10 mg), obtained in Expt. No. 9, was dissolved in ethanol (10 ml). The solution was cooled in an ice-bath and 5 mg of sodium borohydride was added. The mixture was shaken. After some time the solution turned almost colourless. At this stage water was added and the reaction product was extracted with diethyl ether. The ethereal extract was washed with water and dried over anhydrous sodium sulphate.
Fig. 42. Absorption Spectrum of Retinol.

Fig. 43. Absorption Spectra of the SbCl$_3$ products of:
The ether was removed under reduced pressure and the residue was taken in light petroleum.

The light petroleum solution was next transferred to a chromatographic column packed with alumina weakened by the addition of 8 per cent. (v/w) of water. A pale yellow zone was found to be formed at the top of the chromatogram. The chromatogram was developed first with light petroleum and then with a mixture of diethyl ether and light petroleum containing gradually increasing amounts of diethyl ether (1-10%, v/v). The pale yellow zone, however, could not be eluted. The chromatogram was extruded and the material from the yellow zone was eluted with ether. The ether was removed and the residue was dissolved in light petroleum. The spectral characteristics of the substance were examined. Results are as follows:

\[ \lambda_{\text{max.}} = 325 \text{ m\u} \text{ (in light petroleum). (Fig. 42).} \]

Antimony trichloride reaction: \[ \lambda_{\text{max.}} = 615-617 \text{ m\u} \text{(Fig. 43).} \]

Repetition of this experiments showed the results to be reproducible.

11. Anhydroretinol from retinol:

Retinal (50 mg) obtained from retroretinol in Expt. No. 9, was reduced with sodium borohydride in ethanol as described in Expt. No. 10. The reaction product in light petroleum was chromatographed on a column of weakened alumina (10% water; v/w). The chromatogram was extruded and the zone con-
Fig. 44. Absorption Spectrum of Anhydroretinol.
taining retinol was eluted with diethyl ether. After removing the ether by evaporation under reduced pressure the residue (retinol) was treated with 0.033N ethanolic hydrogen chloride (25 ml). The reaction mixture was kept at room temperature for 15 minutes in the dark. The resulting greenish-yellow solution was then neutralised with a suitable amount of sodium bicarbonate and the product was extracted with light petroleum as usual. The extract was concentrated to about 5-3 ml by evaporation under reduced pressure. The solution was next chromatographed on a column of deactivated alumina (10 g; 5% water). A yellow zone passed quickly through the column on development with light petroleum. The percolate was collected and examined with the help of a spectrophotometer.

The ultraviolet absorption spectrum of the solution showed λmax. at 350, 369 and 390 μ with an inflection at 335 μ (Fig.44). Moreover, it produced a blue colour with antimony trichloride reagent and the reaction product exhibited λmax. at 615-617 μ (Fig.43).

These results indicate that the substance produced is anhydroretinol. Reproducible results were obtained on repeating the experiments.

12. Chromatography of a mixture of retinal from retroretinol and that from retinol:

A sample of pure retinal was prepared from retinol by chromatographic oxidation on manganese dioxide (vide Expt. No.8.1, Part I).
A solution of retinal (about 5 mg) in light petroleum (obtained from retroretinol in Expt. No. 9), was mixed with about 5 mg of retinal obtained from retinol and the mixture was chromatographed on a column of deactivated alumina (20 g; 8% water). An orange-yellow zone was found to be formed on the chromatogram. The column was washed first with light petroleum and then with light petroleum containing 2% (v/v) of ether. No separation of the single orange-yellow zone was observed when the column was examined under ultraviolet light. The orange yellow substance was finally eluted with light petroleum containing 2% (v/v) of ether.

The eluate was evaporated to dryness under reduced pressure and the residue was dissolved in light petroleum. The spectral characteristics of the solution were examined. These were:

\[ \lambda_{\text{max.}} \]
\[ 339 \text{ m\mu (in light petroleum)} \]
\[ 380 \text{ m\mu (in ethanol).} \]

Antimony trichloride product: \[ \lambda_{\text{max.}} 660 \text{ m\mu.} \]

The identity of retinal obtained from the two sources, viz., retinol and retroretinol, is thus established from the results of experiments 10-12 recorded above.

13. Action of ethanolic hydrogen chloride on retroretinol:

Retroretinol (10 mg; obtained from retinol) was dissolved in 0.033N ethanolic hydrogen chloride (20 ml) and the reaction mixture was kept at room temperature for 15 minutes
in the dark. Then the reaction product was extracted with light petroleum as described in Expt. No. 9, Part I. The light petroleum extract was next chromatographed on a column of deactivated alumina (5% water). A single yellow zone was found to be formed about 2 cms below the top of the chromatogram. The chromatogram was developed with light petroleum and the eluate was examined spectrophotometrically. The eluate showed no characteristic absorption in the range 270-400 μ of the spectrum.

The yellow zone was eluted with light petroleum containing 10%(v/v) of ether and was found to contain unchanged retroretinol: λmax. 332, 348 and 367 μ; antimony trichloride reaction product, λmax. 580 μ.

This experiment showed that unlike retinol retroretinol could not be dehydrated by ethanolic hydrogen chloride.

14. Identity of retroretinol preparations:

The identity of retroretinol prepared from retinol by the method outlined by Beutel et al. with that prepared by the action of ethanolic hydrogen chloride, is proved as follows:

14.1 Chromatography of a mixture of retroretinol prepared by the different methods:

Retroretinol (10 mg), obtained in Expt. Nos. 1-4, was mixed with retroretinol (15 mg) prepared from retinol by the action of concentrated hydrobromic acid, and the mixture in
light petroleum was chromatographed on a column of deacti­
vated alumina (5% water). The column was developed with light
petroleum and the chromatogram was found to contain a single
yellow zone. This zone neither split up nor passed through
even when developed with a mixture of light petroleum and
ether (98:2). The material from this zone could, however, be
eluted with light petroleum containing 10%(v/v) of ether.

The eluate was evaporated to dryness under reduced
pressure and the residue in light petroleum exhibited \( \lambda_{\text{max.}} \)
at 332, 348 and 367 m\( \mu \).

This experiment showed that the two different retro­
retinol preparations are identical.

14.2 In this experiment a mixture of the esters (acetate) of
the two different retroretinol preparations was chromatogra­
phed on deactivated alumina (20 g; 5% water). A single yellow
zone was found to be formed on the column. The yellow zone
was moving slowly on development with light petroleum and
did not split up into zones but passed through. The eluate
showed \( \lambda_{\text{max.}} \) at 332, 348 and 367 m\( \mu \) and gave a violet colour
with antimony trichloride reagent exhibiting \( \lambda_{\text{max.}} \) at 575 m\( \mu \)
with a minor maximum at 470 m\( \mu \).

Thus, this experiment too supported the view that
the different retroretinol preparations were identical.
14.3 Comparison of the absorption maxima and extinction coefficients of retroretinol preparations:

The absorption maxima and the extinction coefficients are shown in the following table.

Table XXII

<table>
<thead>
<tr>
<th>Substance</th>
<th>Method of preparation</th>
<th>Absorption maxima (mµ)</th>
<th>E(1%,1cm)</th>
<th>SbCl₃-Colours maxima (mµ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retroretinol</td>
<td>By ethanolic HCl</td>
<td>332</td>
<td>1270</td>
<td>580</td>
</tr>
<tr>
<td></td>
<td></td>
<td>348</td>
<td>1593</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>367</td>
<td>1274</td>
<td></td>
</tr>
<tr>
<td>Retroretinol</td>
<td>By conc.HBr</td>
<td>332</td>
<td>1251</td>
<td>580</td>
</tr>
<tr>
<td></td>
<td></td>
<td>348</td>
<td>1579</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>367</td>
<td>1257</td>
<td></td>
</tr>
<tr>
<td>Retroretinyl</td>
<td>By ethanolic HCl</td>
<td>332</td>
<td>1069</td>
<td>575, ~ 470</td>
</tr>
<tr>
<td>acetate</td>
<td></td>
<td>348</td>
<td>1382</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>367</td>
<td>1061</td>
<td></td>
</tr>
<tr>
<td>Retroretinyl</td>
<td>By conc.HBr</td>
<td>332</td>
<td>1055</td>
<td>575, ~ 470</td>
</tr>
<tr>
<td>acetate</td>
<td></td>
<td>348</td>
<td>1367</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>367</td>
<td>1061</td>
<td></td>
</tr>
</tbody>
</table>

The above table indicates that the two retroretinol preparations are identical.

14.4 Comparison of the Infrared spectra of retroretinol preparations:

The infrared spectra of the different retroretinol preparations were compared. The following table showed the different bands (in cm⁻¹) present in the infrared spectra of the retroretinol preparations.
It is evident from the above table that the different retroretinol preparations are identical.

**DISCUSSION**

Results presented in the preceding chapter (Ch. III, part II) show that - of the three different compounds in addition to anhydroretinol, obtained by the treatment of retinol with dry ethanolic hydrogen chloride, the compound showing $\lambda_{\text{max.}}$ at 230 m$\mu$ and forming a pale yellow zone occupying the top position in the chromatogram is kitol. The second yellow zone exhibited $\lambda_{\text{max.}}$ at 332, 348 and 367 m$\mu$.
which was suspected to be rehydroretinol by Barua and Rao is found to be retroretinol.

It has been observed that the yield of retroretinol by this method of preparation is dependent on such factors as (a) concentration of HCl acid used, (b) duration of reaction and (c) temperature.

The optimum conditions for maximum yield of retroretinol from retinol by treatment with dry ethanolic hydrogen chloride determined by careful investigation showed that the best results were obtained by using 0.13N HCl and allowing the reaction to proceed for $\frac{1}{2}$ hours at a temperature lying between 30-35°C. Longer duration of reaction as well as higher temperature were found to produce some other compounds with low extinction coefficients, which resulted in reduced yield of retroretinol. At temperature lower than 30°C also, the yield was poor.

Retroretinol is strongly adsorbed by alumina in the chromatographic column. It is, however, less strongly adsorbed than kitol and retinol. It formed the second zone from the top in the chromatogram, the first being formed by kitol.

The principle of flowing chromatography was applied for the purification of retroretinol. When the crude product was chromatographed on water-deactivated alumina column (plus 10% water) retroretinol could be eluted with light petroleum containing 10 per cent. (v/v) of ether. The ultimate sample of retroretinol thus obtained gave $E(1\%, 1\text{cm})$ values of 1270, 1593 and 1273 at 332, 348 and 357 m\(\mu\), respectively. Retro-
retinol produced a bluish-violet colour with Carr-Price reagent and the antimony trichloride reaction product showed $\lambda_{\text{max}}$ at 580 m$\mu$.

That retroretinol contains hydroxyl group was proved by its behaviour in the chromatographic column where it is strongly adsorbed. The occurrence of a characteristic band at 3400 cm$^{-1}$ in the infrared spectrum of the compound also proved conclusively the presence of hydroxyl group in it.

The hydroxylic nature of retroretinol was further confirmed by the preparation of its ester with acetic anhydride and pyridine. On chromatography, the ester thus prepared passed quickly through the column unlike the parent compound. On saponification with freshly prepared alcoholic KOH the ester was hydrolysed to retroretinol.

Retroretinol thus prepared gave retinal on oxidation with manganese dioxide. The yield is very poor and a maximum yield of about 6% was all that had been achieved by passing retroretinol through a column of manganese dioxide (1:15). Retinal, thus prepared showed $\lambda_{\text{max}}$ at 339 m$\mu$ in light petroleum and 380 m$\mu$ in ethanol and the antimony trichloride colour maximum was at 660 m$\mu$.

The mechanism of formation of retinal from retroretinol has been discussed in Part I, Chapter IV.

The formation of retinal was further confirmed by reduction of retinal with sodium borohydride to retinol: $\lambda_{\text{max}}$ 325 m$\mu$, SbCl$_3$ reaction maximum at 615-617 m$\mu$. Retinol was, in
turn, treated with 0.033N ethanolic hydrogen chloride when anhydroretinol having $\lambda_{\text{max.}}$ at 350, 369 and 390 m$\mu$ (in light petroleum) was obtained.

Unlike retinol, retroretinol is resistant to ethanolic hydrogen chloride. No apparent change in retroretinol was observed when treated with ethanolic hydrogen chloride. This was concluded from the fact that the original material could be completely recovered and showed all the characteristics of retroretinol.

Retroretinol behaved identically in both basic and neutral alumina on chromatography.

A comparative study of retroretinol prepared by the action of concentrated hydrobromic acid on retinyl acetate and that prepared by the treatment of retinol with ethanolic hydrogen chloride was made. Chromatography of a mixture of the two products and their spectral properties (Tables XXII and XXIII) showed that both were identical.

The mechanism of Formation of Retroretinol:

The mechanism of the formation of retroretinol from retinol is considered in the light of the acid-catalyzed dehydration of retinol to anhydroretinol.

The mechanism of the acid-catalyzed dehydration of retinol suggested by Meunier has been discussed in the preceding chapter. The different steps are:
It may so happen that while a very large number of carbonium ions III, that loses a proton to the medium thereby producing anhydroretinol (IV), there is still a small amount of these ions left unchanged. A new carbonium ion (V) probably results from these by a process of rearrangement, which reacts with the OH⁻ ions in the aqueous sodium bicarbonate solution used for neutralisation, forming retroretinol (VI).
An easy allylic rearrangement of the type envisaged in the acid catalyzed dehydration of retinol to anhydroretinol, probably does not take place with facility in the corresponding process of conversion of retinol to retroretinol, which may be responsible for the poor yield of retroretinol.
SUMMARY

1. Retroretinyl acetate was prepared by the treatment of retinyl acetate with concentrated hydrobromic acid at 0°C for 30 seconds. The yield is satisfactory and a maximum yield of about 86% was obtained.

2. Retroretinyl acetate thus prepared is purified by flowing chromatography and values of 1069, 1367 and 1061 are obtained for the purified sample at the points of maximum absorption, viz., 332, 348 and 367 μm, respectively in light petroleum.

3. The ester prepared by the above method is characterised spectroscopically (both ultraviolet and infrared spectra).

4. The corresponding alcohol of the ester is obtained by hydrolysing the ester with freshly prepared alcoholic KOH. The alcohol is characterised from a study of its chromatographic behaviour and ultraviolet and infrared spectra. Unlike retinol retroretinol is found to be resistant to ethanolic hydrogen chloride.

5. Retroretinol obtained from its ester, on oxidation with manganese dioxide gives retinal, which is characterised spectrophotometrically and also by chromatographing a mixture containing an authentic sample of retinal prepared from retinol.

6. Retinal, on reduction with sodium borohydride yields retinol; λmax 325 μm, SbCl₃ reaction maximum 617 μm.
Retinol obtained from retinal, gives anhydroretinol on treatment with ethanolic hydrogen chloride.

7. Retroretinol behaves identically when chromatographed on basic as well as neutral alumina.

8. Retroretinol is prepared from retinol by the treatment with 0.033N ethanolic hydrogen chloride at room temperature for 40 minutes. The yield is however poor, 1.8-2%.

9. A better yield of retroretinol by the above method is obtained by treating retinol with 0.13N ethanolic hydrogen chloride for 40 minutes at room temperature. Yield 5.6 per cent.

10. The yield of retroretinol is further increased by increasing the time of reaction to $1\frac{1}{2}$ hours with other conditions remaining the same. In this case yield is 10.0%.

11. A maximum yield (10.1%) of retroretinol is achieved when retinol is treated with 0.13N ethanolic hydrogen chloride for $1\frac{1}{2}$ hours at 30-35°C.

12. Retroretinol prepared by this method is purified by flowing chromatographic method. The best sample prepared gave E(1%,1cm) values of 1270, 1593 and 1274 at 332, 348 and 367 μμ, respectively.

13. Retroretinol thus prepared is characterised spectrophotometrically (ultraviolet and infrared).

14. The hydroxylic nature of retroretinol is confirmed by a study of its i.r. spectrum which gives a band at 3400 cm⁻¹ and also by its behaviour on chromatographic column.
where it is adsorbed strongly. Retroretinol is resistant to the action of ethanolic HCl and the recovered material exhibited all the properties of retroretinol.

15. On treatment with a mixture of acetic anhydride and pyridine, retroretinol produces the corresponding acetate, which is characterised spectrophotometrically (ultraviolet and infrared).

16. Retroretinol gives retinal, on oxidation with manganese dioxide. A maximum yield of about 6% is obtained when oxidised with a 15 fold quantity of manganese dioxide.

17. On reduction with sodium borohydride retinal thus produced gives retinol which, in turn, gives anhydroretinol on treatment with 0.033N ethanolic hydrogen chloride.

18. Chromatography of a mixture of retroretinol prepared by these two different methods proves that both are identical.

19. The identity of these two products is proved by comparing the spectral properties of one with those of other.
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RETINAL FROM RETRORETINOL

Retroretinol, generally found in deteriorated samples of multivitamin preparations, can be prepared by treatment of retinyl acetate with concentrated hydrobromic acid. Barua and Rao reported the formation of a compound whose absorption spectrum resembled that of retroretinol. Although there is indication of in vivo conversion of retroretinol to retinol, there is no report till now of the chemical conversion of retroretinol to retinol. In this communication we report a new procedure for the preparation of retroretinol and the conversion of retroretinol to retinal.

Preparation of Retroretinol.—200 mg. of retinol was treated with an alcoholic solution of dry hydrogen chloride (0.13 N; 100 ml.) and allowed to stand for 40 minutes in the dark. The reaction mixture was then neutralized with sodium bicarbonate solution and the product was extracted with light petroleum. Chromatography of the product on water-deactivated alumina (5% water, v/w) resulted in the separation of four zones. The main greenish-yellow zone containing anhydroretinol passed quickly through the column. The second yellow zone was eluted with light petroleum containing 1% (v/v) of ether. The substance from this zone exhibited $\lambda_{max}$ at 330 μm (light petroleum). The third yellow zone was eluted with light petroleum containing 5% (v/v) of ether. The substance from this zone showed $\lambda_{max}$ at 367, 348 and 332 μm (solvent: light petroleum). The fourth zone which was eluted, after extrusion, with ether contained a substance that exhibited $\lambda_{max}$ at 290 μm in light petroleum.

The substance eluted from the third zone was further purified by chromatography and the pure compound exhibited $\lambda_{max}$ at 367, 348, and 332 μm with E (1%, 1 cm.) values of 1274, 1593, and 1270, respectively, at the points of maximum absorption in light petroleum. It produced a bluish-violet colour with antimony-trichloride reagent showing $\lambda_{max}$ at 580 μm. In the I.R. spectrum it showed band at 3400 cm$^{-1}$ characteristic of the –OH group. The yield of retroretinol was 10-1%.

The identity of retroretinol prepared as above was further confirmed by preparing retinyl acetate. Retroretinol was heated with acetic anhydride in pyridine at 50° C. for 30 minutes, the product was extracted with light petroleum and then chromatographed on a column of water-deactivated alumina (5% water; v/w). Retroretinyl acetate ($\lambda_{max}$ 367, 348 and 332 μm in light petroleum) was eluted with light petroleum. It produced a violet colour with Carr-Price reagent showing $\lambda_{max}$ at 375 μm with a minor maximum at 470 μm.

The ultra-violet and I.R. spectra and SbCl$_3$ colour absorption maxima of retroretinol and retroretinyl acetate prepared by this new procedure and those prepared by the procedure outlined by Beutel et al. were found identical in all respects. Further, co-chromatography of samples of either retroretinol or retroretinyl acetate prepared by these two procedures, on a column of water-deactivated alumina showed that the compounds were identical. Only a single zone was formed on the chromatogram and prolonged development did not effect separation of the zone.

Conversion of Retroretinol into Retinal.—20 mg. of retroretinol obtained by the above procedure was dissolved in 10 ml. of light petroleum and passed through a column of manganese dioxide (300 mg, precipitated, B.D.H.). The filtrate was concentrated and chromatographed on a column of water-deactivated alumina (8% water, v/w). The substance from the main zone which was eluted with light petroleum or better with light petroleum containing ether (1-2%, v/v), was found to be retinal (1-2 mg.). $\lambda_{max}$ 369 μm in light petroleum and 380 μm in ethanol; SbCl$_3$ colour maximum at 660 μm.

Retinal thus obtained was found to be identical with an authentic sample prepared from retinol by the method of Ball, Goodwin and Morton in their ultra-violet and SbCl$_3$ colour-visible spectra. Mixed chromatography of the two retinal samples did not result in the separation of zones.

Retinal (10 mg.), prepared from retroretinol, was reduced with NaBH$_4$. The reduced product after chromatographic purification was found to be retinol (7-56 mg.), $\lambda_{max}$ 325 μm; SbCl$_3$ colour $\lambda_{max}$ 617 μm. Treatment of retinol with 0.033 N ethanolic hydrogen chloride followed by purification by chromatography gave anhydroretinol, $\lambda_{max}$ 390, 369 and 350 μm; SbCl$_3$ colour $\lambda_{max}$ 617 μm.
Retinal was also obtained in 6% yield when retroretinol, prepared by the procedure of Beutel et al., was oxidised with MnO₂.

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