sor of ethylene. The hypothesis is advanced that nickel inhibits either the synthesis of SMM or the transaminase of homocysteine or its precursors. Synthesis of cysteine [7] and hence homocysteine occurs in leaves.

If vase solutions containing nickel ions inhibit the leaf synthesis of cysteine and homocysteine that explains why our leafy *Chrysanthemum* blooms responded to nickel by delayed flower senescence, whereas the leafless blooms of *Papaver nudicaule* did not.

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# Chemical Identity of the Chromophores of Fly Visual Pigment

K. Vogt and K. Kirschfeld
Max-Planck-Institut für biologische Kybernetik, D-7400 Tübingen

Since Wald's discovery of retinal as the chromophoric group of the visual pigment in vertebrates [1] it has become generally accepted that with the exception of the Vitamin A2-based porphyropsins [2] in some fishes and amphibia, retinal is universal in the animal kingdom. This view is further reinforced by the more recent finding of retinal in a quite distant form of life – the Halobacteria – where it is the chromophore of bacteriorhodopsin [3]. With evidence resting mainly on spectrophotometric data, it has also generally been accepted that retinal is the chromophore of the frequently investigated visual pigment in flies [4]. Here we present evidence that the visual pigment chromophore in fly photoreceptors is not retinal but 3-hydroxy-retinal. In most photoreceptors of the fly there is an additional "sensitizing pigment" which absorbs uv quanta and transfers the energy to the visual pigment [5]. The sensitizing chromophore, which explains the high uv sensitivity in these receptors, is identified as 3-hydroxy-retinol.

We isolated an aldehyde from fly eyes (*Calliphora erythrocephala*), the oxime derivative of which is considerably more polar than retinal oxime. This aldehyde was identified as the visual pigment chromophore due to its absence in blind (carotenoid-deprived) flies and the occurrence of distinct stereoisomers, depending on the colour of light adaptation preceding the extraction [6]. The uv extinction spectrum of the oxime of the chromophore aldehyde is virtually identical with that of retinal oxime, thus not contradicting the former spectral evidence for retinal. Furthermore, we showed that xanthophylls with 3-hydroxy groups (zeaxanthin, lutein) in flies can be used as precursors for the chromophore, and that $C_{40}$ carotenes such as $\beta$-carotene can be hydroxylised to the xanthophylls cryptoxanthin and zeaxanthin in the metabolism of flies [6, 7].

Besides the polar aldehyde a still more polar compound exhibiting strong whitish fluorescence can be isolated. This is also considerably reduced in flies with low visual sensitivity. The chemical relationship between these compounds can be shown by reduction of the aldehyde with $NaBH_4$ in ethanol: rechromatography of the reaction product shows its identity with the fluorescing compound extracted from the eyes, which therefore can be regarded as the alcohol of the chromophore aldehyde. The uv extinction spectra of both, the isolated aldehyde and alcohol (Fig. 1) are indistinguishable from those of all-trans-retinal and retinol, respectively. This indicates that the conjugated system is of the same length as in retinal (5 double bonds) with a ring double bond conjugated to the chain ($\beta$-ring).

The number of hydroxy groups present was determined by esterification under conditions in which primary and secondary hydroxy groups react [8]. When the time course of acetylation (acetic anhydride in dry pyridine, room temperature) is followed by chromatographic inspection the original aldehyde is found to produce a single and considerably less polar spot, whilst the alcohol produces two. The identity of the reaction products as acetylesters was confirmed by saponification which yielded the original compounds (Fig. 2). Thus we conclude that a single hydroxy group is present in the chromophore aldehyde and two in the chromophore alcohol.

The possible positions of the hydroxy groups can be narrowed by testing their ability to form methylethers (by hydrogen chloride in methanol [9, 10], chromatographical inspection). Under conditions in which the secondary hydroxy groups in allyl position of isozeaxanthin ($\beta,\beta$-carotene-4,4′-diol, obtained by reduction of canthaxanthin from Hoffman-La Roche) and lutein ($\beta,\varepsilon$-carotene-3,3′-diol, isolated from spinach) form methylethers, the chromophore aldehyde is not methylated as is the case with the non-allylic secondary hydroxy groups of zeaxanthin ($\beta,\beta$-carotene-3,3′-diol, isolated from maize) and $\beta,\beta$-carotene-2,2′-diol (isolated from stick insects [11]). We therefore may exclude an allylic hydroxy group

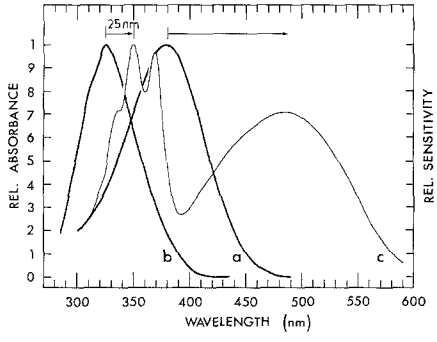



Fig. 1. Absorbance spectra of the chromophore aldehyde (*a*, $\lambda_{max}$ 380 nm) and the chromophore alcohol (*b*, $\lambda_{max}$ 325). The thin line (*c*) represents the spectral sensitivity (redrawn from [13]) of the most common fly photoreceptor (R1–6). Arrows indicate the shift between the spectra of the two chromophores in ethanol and the corresponding spectral sensitivity peaks

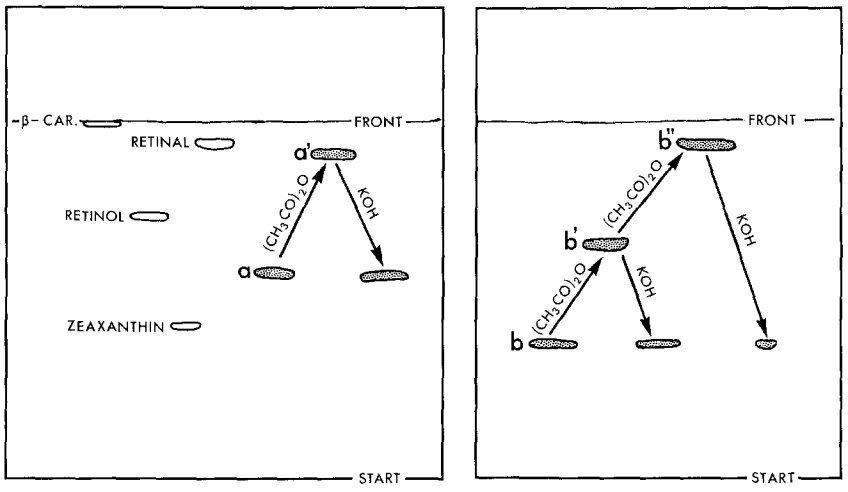
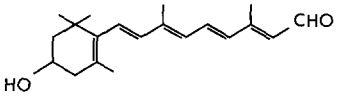


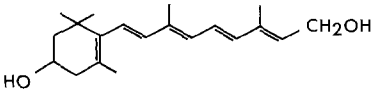

Fig. 2. Thin-layer chromatograms (silica gel with concentrating zone, Merck; solvent: dichloromethane, ethyl acetate, 7+3) of the chromophore aldehyde (*a*), the chromophore alcohol (*b*) and the respective acetylesters (*a'*, *b'*, *b''*). Chromophores were isolated from 2000 fly eyes and redissolved. A part of each of the resulting solutions was kept for initial references (*a* and *b*) and the remainder acetylated (acetic anhydride in dry pyridine, 20 °C, 30 min). The resulting monoesters (*a'* and *b'*) and the diester (*b''*), which are less polar than the native chromophores, were again isolated and redissolved. The solutions were again divided and one part saponified in order to demonstrate the identity of the reaction products as esters. Then all solutions (*a*, *a'* and the saponification product, and *b*, *b'*, *b''* and the saponification products) were simultaneously applied to the plate. The result indicates the presence of one hydroxy group in the chromophore aldehyde (*a*) and two in the chromophore alcohol (sensitizing pigment). Reference substances: all-trans-retinal, all-trans-retinol, β-carotene (Sigma); zeaxanthin (from maize)

as e.g. in the 4-hydroxy-retinal (which is able to bind to bacteriorhodopsin [12]) and favour a C3 or C2 position. The chromophore alcohol exhibits a single methylation product as does retinol under the same conditions.

From this evidence, and the fact that the 3-hydroxy compound zeaxanthin can serve as metabolic precursor for these compounds, we derive the formulae of 3-hydroxy-retinal and 3-hydroxy-retinol, respectively (Fig. 3).




3 - HYDROXY-RETINAL




3 - HYDROXY- RETINOL

Fig. 3. Formulae for the covalently bound "normal" chromophore (3-hydroxy-retinal) and the sensitizing chromophore (3-hydroxy-retinol) of the fly visual pigment (xanthopsin)

The identity of the aldehyde with the "normal" chromophore of the visual pigment is well substantiated [6]. With respect to the diol, no other compound is extractable from the eyes, which fulfills the prerequisites for the sensitizing pigment. These are: a) the occurrence must depend on carotenoid supply (as does the uv extinction in single rhabdomeres [13]), b) it should exhibit strong fluorescence and c) absorb in the appropriate spectral range. At first sight, the last condition does not seem to be met by the 3-hydroxy-retinol since the isolated chromophore has a peak absorbance at 325 nm while the uv sensitivity of fly photoreceptors (R1–6) is maximal at 350 nm and exhibits a fine structure with two secondary peaks at 369 and 332 nm [14] (Fig. 1). This discrepancy may be explained, however, by analogy with retinol, resulting in a shift of +25 nm and a fine structure if the ionon ring is approximately coplanar with the polyene chain [15].

Such a coplanarity can be achieved either by a retro structure (C6–C7 double bond) or by binding of retinol to a protein. Since a retro molecule can be excluded – we cannot detect a fine structure in extinction even in raw extracts – the latter possibility is most favourable. The binding of retinol and the stabilisation of its β-ring with respect to the chain has been attributed to a kind of hydrophobic interaction in the retinol protein complex [16]. For 3-hydroxy-retinol an alternative or additional explanation is suggested by its formula: the ring and the chain hydroxy groups may form hydrogen bonds with polar groups of the opsin, thus contributing to the stability of a coplanar conformation. The fact that the diol is easily removed from photoreceptor membranes by polar solvents such as insect Ringer solution supports the suggestion of hydrogen bonding. Some form of fixation of the sensitizing molecule to the opsin has in fact already been inferred from the high quantum yield of sensitization ($\geq 0.8$), which can only be explained by a distance of less than 2.5 nm between the two chromophores [17].

All naturally occurring retinal-based visual pigments are usually called rhodopsins and all 3-dehydro-retinal pigments are termed porphyropsins [18]. Since the visual pigment of flies has a chromophore whose sum formula is not that of retinal, a new name seems justified. We suggest xanthopsin [6], since the chromophore can be derived from oxygenated carotenoids i.e. xanthophylls.

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