(15) The calculated intensity ratio of (M + 2)/(M + 4) of **3** if these oxygens came from two O_2 molecules is 2.63 (found 2.39), whereas, if from one O_2 molecule, it is ≤ 0.07.

(16) For example, in our working hypothesis i for ring formation, the C-4 hydroxyl would be a necessary result, but not the C-7 hydroxyl; in the alternative hypothesis ii, the C-7 hydroxyl could be a necessary result, but not the C-4
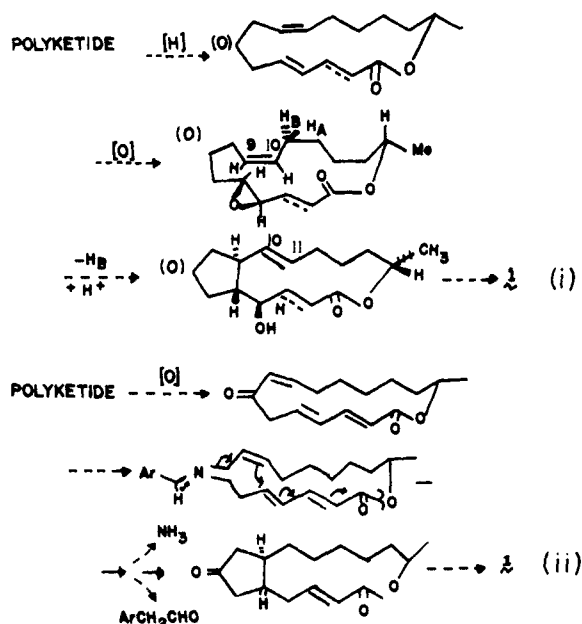
hydroxyl; in the proton-initiated analogue of hypothesis i, neither the C-4 nor C-7 hydroxyl would be a necessary result.

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A Nonbleachable Rhodopsin Analogue Formed from 11,12-Dihydroretinal

Sir:

It is now commonly accepted[1] that the chromophore of the visual pigment rhodopsin consists of the protonated Schiff base[2] of 11-*cis*-retinal (**1**) bound to an ϵ-amino group of lysine[3] of opsin, and that light triggers a series of reactions the terminal products of which are *all-trans*-retinal and opsin. Although the detection of the initial photolysis product, bathorhodopsin,[4] and the room temperature[5] and low temperature measurements[6] of bleaching have been achieved, the nature of complex transformations and spectral changes are largely unsolved and remain to be the central problems in understanding the visual process on a chemical basis. A major factor which renders studies of rhodopsin difficult is its great lability toward light and heat. In conjunction with our studies on model retinals and rhodopsins formed therefrom,[7] we report the preparation of the first nonbleaching rhodopsin and some spectral data which have direct bearing in clarifying the chemistry of rhodopsin.

The synthesized chromophore was *all-trans*-11,12-dihydroretinal (**2**), which, in view of the flexibility around the C_{11}–C_{12} bond, could conceivably bind to opsin; this was found to be the case. This chromophore is pertinent since (i) there is no cis/trans isomerism around the 11–12 bond and hence the rhodopsin analogue would be "nonbleachable"; (ii) owing to the separation of the chromophore into triene and enal moieties, spectroscopic properties of the pigment would contribute to clarifying the cause of the enigmatic red shift accompanying its formation.
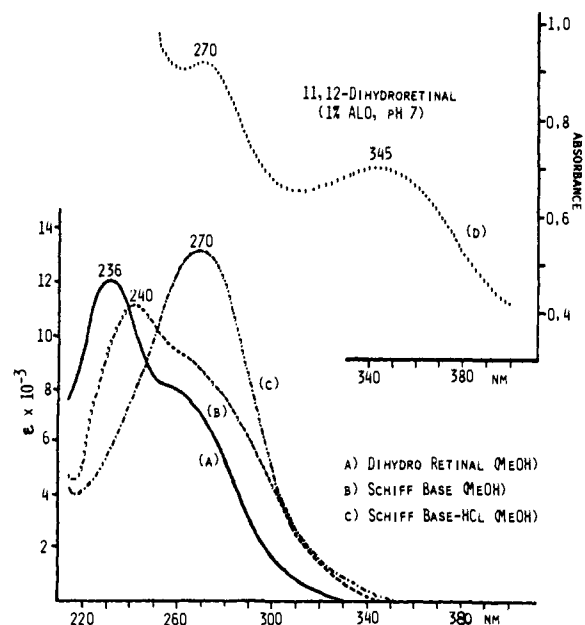

Figure 1. Absorption spectra: Curve a: aldehyde **2** in methanol; curve b, Schiff base **6** in methanol; curve c, protonated Schiff base **7** in methanol; curve d, pigment from 11,12-dihydroretinal **2** and bovine opsin in ALO, pH 7.0.

Table I. Absorption Spectral Data of 11-*cis*-Retinal and Dihydroretinal

	Dihydroretinal, in MeOH	11-*cis*-Retinal, in EtOH
Aldehyde	236 (*12 000*),[a] 255 (8 000)	375 (20 000)
Schiff base with BuNH$_2$	240 (*11 000*),[b] 255 (8 000)	350 (29 000)[b]
Schiff base–HCl	270 (*13 000*)[c]	440 (34 000)[c]
Pigment	345 (in ALO, pH 7.4)	500 (in ALO, pH 7.4)[d]

[a] Maxima due to the enal chromophore are italicized. [b] Prepared by keeping the solution of aldehyde in neat amine over molecular sieves, −20 °C, 12 h, under nitrogen in the dark, blowing off excess amine, and dissolving residue in MeOH—*strictly anhydrous conditions.* [c] Prepared by bubbling dry HCl gas into methanol solution of the Schiff base at −78 °C, under argon in the dark. [d] Data for bovine opsin.

11,12-Dihydroretinal (**2**) was prepared by the Emmons reaction between the ethyl phosphonate reagent derived from the chloro ketal **3** and β-ionone. The ketal was hydrolyzed to the dihydro C_{18} ketone **4** with 10% HCl/THF, and this was submitted to a second Emmons reaction with ethyl (2-carbethoxy)phosphonate to give the ethyl ester **5**, which was converted to the aldehyde by reduction with diisobutylaluminum
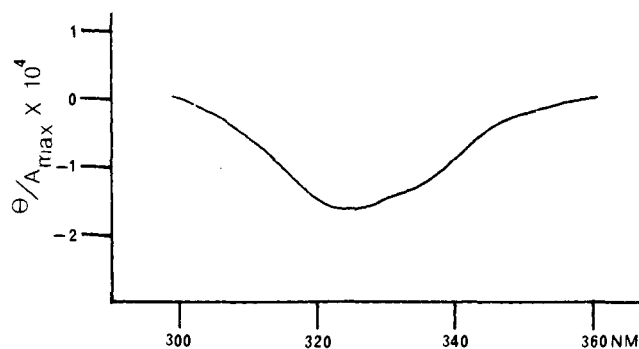

1 CHO

2, R = CHO
5, R = CO$_2$Et
6, R = CH=N–Bu
7, R = CH=$\overset{+}{N}$H · Bu

3

4
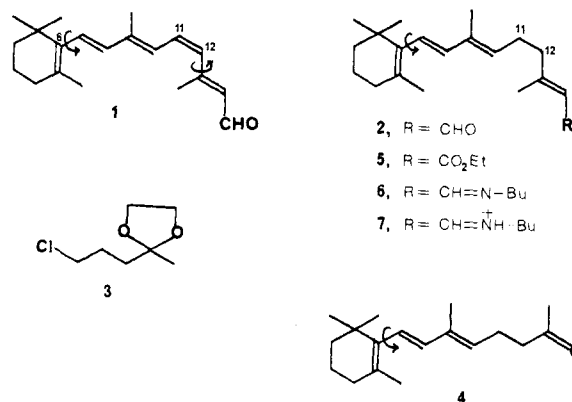
Figure 2. Circular dichroism curve of the pigment formed from 11,12-dihydroretinal **2** and bovine opsin in 1% ALO, pH 7.

hydride and subsequent oxidation with manganese dioxide. Separation by analytical HPLC, μ-Porasil, 10% ether in hexane, of the major isomer of the four-component mixture afforded pure all-trans-11,12-dihydroretinal (**2**) as characterized by ^{1}H NMR and ^{13}C NMR spectra. The chromophore is extremely unstable to traces of acid, base, or oxygen, and starts to deteriorate in a few days even when stored under nitrogen in the dark at -20 °C. Because of its lability, the chromophore should be kept as ester **5**, and converted to the aldehyde and purified by HPLC immediately prior to usage, or stored at -65 °C under argon.

Incubation of this dihydroretinal with bovine opsin suspension[8,9] for 3 h at 37 °C resulted in the appearance of a peak above 300 nm. The product was then purified by calcium phosphate chromatography, 1% Ammonyx LO.[10] Control incubations employing only opsin or the chromophore gave no peaks above 300 nm and hence the new peak at 345 nm (Table I, Figure 1), which was accompanied by a CD extremum (Figure 2),[11] must be due to pigment formation. As expected, the UV spectrum was changed neither by exposure to room light for 10 h nor by direct irradiation for 0.5 h at room temperature with a 275-W sun lamp, and hence the pigment is "nonbleachable".

Absorption data for the dihydro chromophores **2, 6, 7,** and pigment are shown in Table I together with the corresponding data for 11-cis-retinal and rhodopsin (see also Figure 1). The spectra of the protonated Schiff bases (SBH$^+$) were measured in the "leveling" solvents ethanol and methanol because in these solvents the UV spectra of SBH$^+$ are insensitive to the method of preparation and the counteranion.[12]

One of the most important unsolved problems in vision chemistry concerns the large red shifts seen in the various rhodopsins, which absorb from 460 to 560 nm depending on the opsin,[13] as opposed to the 440-nm value for SBH$^+$ (Table I). Numerous models and theoretical calculations have been forwarded to account for this. For example, the following electrostatic interactions between SBH$^+$ and the protein receptor site have been proposed: (i) between C=N$^+$H and a nearby counterion;[14] (ii) between C=N$^+$H, a nearby anion, and an additional anion close to the trimethylcyclohexene ring;[15] (iii) between delocalized positive charge and nucleophilic groups along the side chain;[16] (iv) between C=N$^+$H and polarizable aromatic amino acid residues,[17] etc.

However, it is remarkable that, in spite of the much shorter chromophore of dihydroretinal, the red shift between SBH$^+$ and pigment is *larger* than in the case of natural rhodopsin, i.e., 270 nm to 345 nm (or 8051 cm^{-1}) in contrast to 440 nm to 500 nm (or 2727 cm^{-1}) (Table I).

None of the above theories can satisfactorily rationalize this dramatic shift of 8051 cm^{-1}. It is quite possible that several effects are contributing simultaneously; it is also possible that the situation encountered in the dihydro pigment differs from normal rhodopsins. However, there undoubtedly exists within the protein cavity an environment which induces the short dihydro-SBH$^+$ chromophore to undergo a further red shift of 75 nm. Preparations of other dihydroretinals,[18] comparisons of spectral properties of their pigments formed from bovine and other opsins, and theoretical calculations based on the results[19] will contribute to an understanding of this problem.[20]

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(18) Preparation of 7,8-, 9,10-, and 13,14-dihydroretinals and their pigments is in progress.

(19) Calculations are being carried out by Professor B. Honig, Hebrew University, Israel.

(20) The studies were supported by NIH Grants EY 01253, EY 01811, and a grant from Hoffmann-La Roche, Inc. We also acknowledge the help of Dr. F. Pilkiewicz and M. Pettei in the HPLC purification of the dihydroretinal.

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A Novel Phenol–Benzene C–C Coupling Reaction. An Acid-Catalyzed Reaction of N-Acyl-O-arylhydroxylamines with Benzenes

Sir:

The reaction of N-phenylhydroxylamine[1,2] (**1**, X = NH; Y = OH), phenylhydrazine[3] (**2**, X = NH; Y = NH$_2$), and related compounds[3–5] with benzene give aminobiphenyls (eq 1) or their derivatives, and may involve a positively charged