Communications to the Editor

13-(Trifluoromethyl)retinal Forms an Active and Far-Red-Shifted Chromophore in Bacteriorhodopsin

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In bacteriorhodopsin (BR, MW = 26 000) of *Halobacterium halobium* the energy of incident light drives a photochemical cycle, to which a proton transport process across the cell membrane is coupled; i.e., BR functions as a light-driven proton pump.[1] This membrane protein contains a retinal molecule bound to the ϵ-amino group of a lysine residue of the protein via a protonated Schiff base (SBH^+).[2,3]

all-trans-Retinal in organic solvents absorbs light around 380 nm. Upon formation of a SBH^+ with amines such as butylamine the λ_{max} shifts to 440 nm (ethanol), whereas association of retinal with bacterioopsin results in a further red shift to 570 nm. Clearly, the environment of the protein's binding site induces this additional bathochromic displacement of 130 nm.

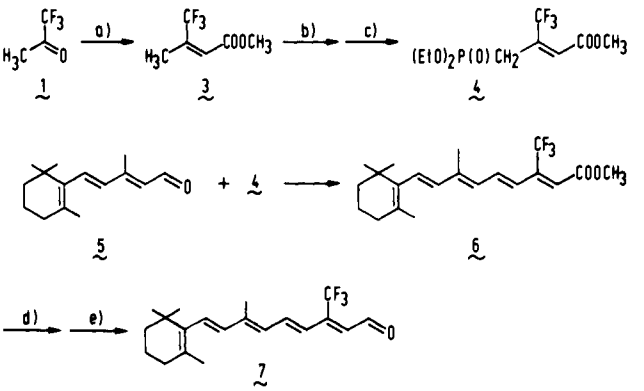
Early work has used retinylidenealkylammonium salts as model compounds and has shown that the distance between the positively charged nitrogen and the negatively charged counterion regulated by the ionic radius of this anion influences the absorption maximum of the model compounds. Theoretical calculations showed that a displacement of the negative charge by 1050 pm would cause an absorption maximum at 570 nm in chloroform as solvent.[4] Such a distance could be produced and maintained by the protein's tertiary structure around the binding site of retinal via charged groups, e.g., carboxyl residues.

In the case of BR the influence of negative charge distribution on the absorption maximum is nicely demonstrated by acidification, which leads to a further red-shifted species with a λ_{max} of 605 nm.[5-7] This transition has a pK around 3 and has been interpreted as the protonation of the counterion of the positively charged nitrogen, thereby changing the negative charge distribution of the protein.[8]

More recently, models have been proposed which place two negative charges strategically around the retinal moiety to account for the absorption maxima of retinal–protein complexes. One of these models, the so-called external two-point charge model, assumes one negative charge at a distance of about 300 pm from the nitrogen atom of the SBH^+ and a second negative charge at a distance of about 350 pm above the plane of the cyclohexene ring of retinal in BR, which is assumed to induce the red shift.[9,10] These models rest on the experimentally observed "opsin shifts" which take place upon the association of bacterioopsin (BO) with a series of dihydroretinals (cf. ref 9). A reasonable fit of the theoretically calculated with the experimentally obtained data was found.

A different approach to analyze the negative charge distribution around the retinylidene moiety is the use of retinal analogues which have a changed electronic structure due to various substituents but have nearly identical molecular shape, thereby not disturbing the binding site's structure but inducing a changed interaction between the negative charges of the protein and the SBH^+. As an example of this class we have synthesised 13-(trifluoromethyl)retinal (**7**) as shown in Scheme I.

Scheme I



^{1}H-NMR data of **7**

chemical shift (ppm)						
H_7	H_8	H_{10}	H_{11}	H_{12}	H_{14}	H_{15}
6.42	6.17	6.21	7.20	6.90	6.29	10.14
coupling constants (Hz)						
$^3J_{7,8}$	$^3J_{10,11}$	$^3J_{11,12}$	$^3J_{14,15}$			
15.9	10.0	15.9	6.6			

a) $(EtO)_2P(O)CH_2COOCH_3$, **(2)**, THF, NaH; b) NBS, CCl_4, rflx; c) $(EtO)_3P$, 2h rflx; d) DIBAH, C_6H_6, RT; e) MnO_2, CH_2Cl_2, RT.

Wittig–Horner reaction of 1,1,1-trifluoroacetone (**1**) with methyl (diethylphosphonato)acetate (**2**) provided methyl 3-(trifluoromethyl)crotonate (**3**) which by NBS bromination followed by Arbusov reaction with triethyl phosphite was converted to the phosphonate **4**. A Wittig–Horner reaction of **4** with β-ionylideneacetaldehyde (**5**, C_{15} aldehyde, prepared from β-ionone) provided methyl 13-(trifluoromethyl)retinoate (**6**), which after reduction and reoxidation furnished the desired **7**. The structure of **7** and intermediates **2–6** formed during the synthesis, which were purified by HPLC, were proven by the usual spectroscopic methods; pertinent data from the particularly informative 400-MHz ^{1}H

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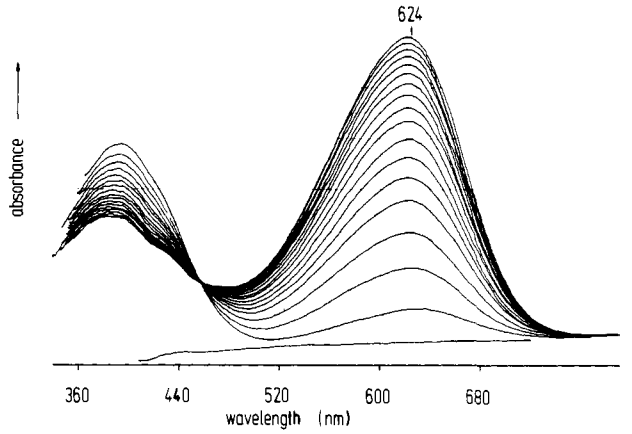

Figure 1. Reconstitution of 13-(trifluoromethyl)retinal-BR. Spectra were recorded continuously on an Aminco DW-2 spectrophotometer.

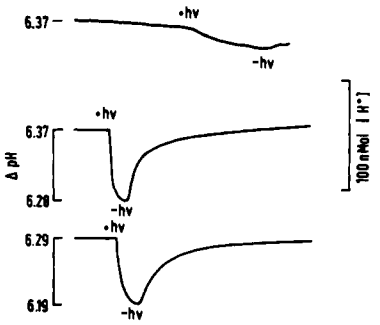

Figure 2. pH response of a 13-(trifluoromethyl)retinal-containing cell vesicle preparation: top, control with retinal-free BO; middle, BR containing (trifluoromethyl)retinal; bottom, BR containing retinal. The ratio of 13-(trifluoromethyl)retinal-BR to retinal-BR, as determined spectroscopically, was about 1:0.7; in both cases the retinals were added in amounts slightly less than stoichiometric.

NMR spectrum, supporting the all-trans configuration of **7**, are also given in Scheme I.[11] All chemicals used for the synthesis were of analytical grade.

When **7** (λ_{max} 390 nm, ethanol) is converted to the SBH^+ with *n*-butylamine, a bathochromic shift to 460 nm is measured. Upon addition of **7** to an aqueous suspension of BO at 15 °C which had been isolated from retinal (–) *H. halobium* mutants (JW 5) the absorption at 390 nm disappeared, and immediate formation of a blue species with an absorption at 624 nm took place with a half-time of formation of approximately 5 min (Figure 1). This λ_{max} constitutes the largest red shift so far observed for a pentaene retinal-containing chromoprotein.

To test the proton-pumping facilities of this new artificial BR, we incorporated **7** into BO-containing vesicles, which were obtained by sonication of *H. halobium* mutant cells deficient in retinal biosynthesis as described in ref 12. Upon reconstitution the same 624-nm chromophore was formed. After being placed in a thermostated (15 °C) cuvette, the vesicle suspension was left in darkness to stabilize the pH. Then the cuvette was illuminated with light from a slide projector, filtered through a cutoff filter (GG 495 nm, Schott), and the pH response monitored (Figure 2). Compared to retinal-containing vesicles, the extent of acidification was about 70%, indicating a proton-pumping activity of 13-(trifluoromethyl)retinal comparable to that of retinal.

The dramatic influence of the trifluoromethyl group on the bathochromic shift ("opsin shift"; cf. ref 9) demonstrates that the electronic properties of the C-13 substituent of retinal do have a pronounced effect on the absorption characteristics of retinals in BO.

(11) The configuration of the 9,10- and 13,14-double bond was confirmed by comparison with data given in the literature: Patel, D. J. *Nature (London)* **1969**, *221*, 825–828.

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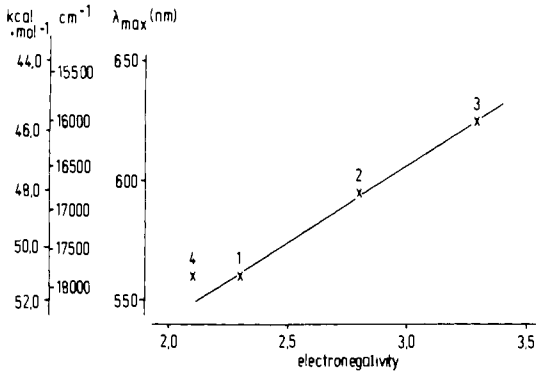

Figure 3. Relationship between the λ_{max} of BR's and the electronegativity of the C-13 substituent of the incorporated retinals (1, CH_3; 2, Br; 3, CF_3; 4, H). The λ_{max} value for 2 (Br) was taken from ref 10.

We note an interesting linear relationship between the electronegativity of the C-13 substituents methyl, bromine, and trifluoromethyl, which possess similar van der Waals radii of about 220 pm, and the absorption shifts of the corresponding BR's, (Figure 3).

The importance of steric identity of the retinal analogues is demonstrated by 13-demethylretinal (H as the C-13 substituent, van der Waals radius of 120 pm), which does not fit into the correlation in Figure 3.

Obviously, the energy difference between the ground and the excited states of retinal in BR strikingly depends on the electron-withdrawing effect of the substituent at position 13. It will be of interest to show by comparison with, for example, 9-(trifluoromethyl)retinal, whether the 13-position of the polyene chain plays a special role in the absorption properties in BR. Furthermore, theoretical analysis of the absorption spectra of these BR analogues will be helpful to check on the charge distribution models already suggested.

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Synthesis of Stabilized Phenyltin(II) Compounds: Inhibition of the Conversion to Tin(IV) by Ortho Substitution[1]

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We have synthesized the first example of an organotin(II) compound with phenyltin(II) σ bonds.

During the past century many reports appeared describing what were believed to be diorganostannylenes.[3-5] Diphenyltin, I, was

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