Interaction of a Conformationally Rigid Analogue of Retinal with Bacterio-opsin

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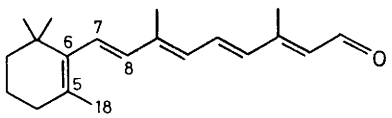
The naphthyl analogue of retinal (**2**) combines with bacterio-opsin to produce an artificial pigment absorbing at λ_{max} 504 nm; this suggests that in the native pigment, all-*trans*-retinal may be held around the 6,7-bond in a planar ring–chain conformation.

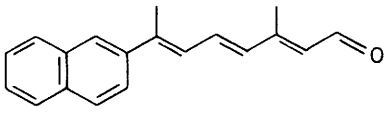
Isomers of retinal (**1**) serve as prosthetic groups for two classes of biologically important proteins, namely, rhodopsins and bacteriorhodopsin. The most extensively studied member of the rhodopsin family is the visual pigment present in the rods of bovine retinae. Bovine rhodopsin has been shown to contain 11-*cis*-retinal bound *via* a Schiff-base linkage[1,2] to the ϵ-amino group of a specific lysyl residue[3] of the apoprotein, opsin.

Bacteriorhodopsin, the major constituent of the purple membrane of *Halobacterium halobium*, functions as a light-driven proton pump,[4] and has all-*trans*-retinal as its chromophoric group also linked to a lysine[5] (Lys-216[6–8] in the primary sequence).

All-*trans*- as well as 11-*cis*-retinal absorbs at *ca.* 390 nm; however, the animal and bacterial rhodopsins derived from them have absorption maxima ranging from 460 to 580 nm. The physico-chemical interactions between the protein and the chromophore which induce this remarkable red-shift have been the subject of much inquiry and speculation during the last two decades.



(1)



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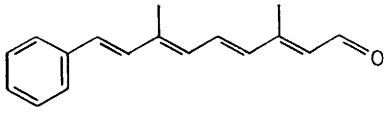


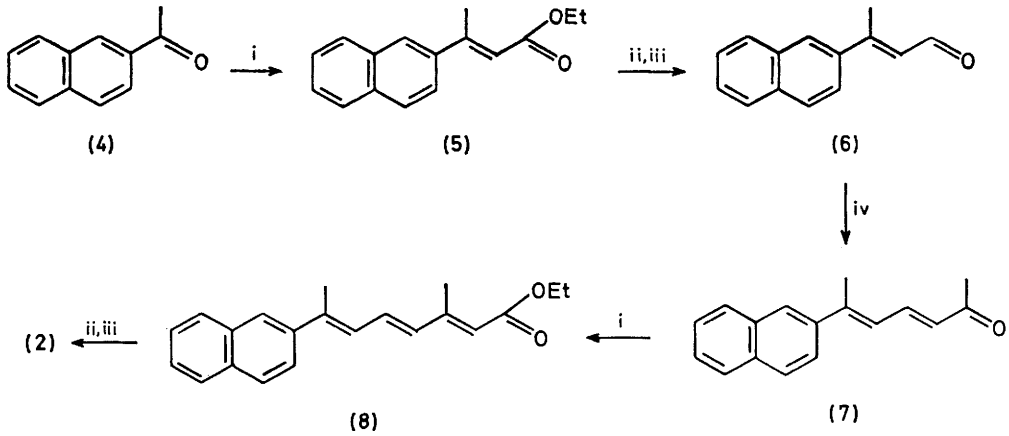
(3)

Table 1. λ_{max}/nm of bacteriorhodopsins and related compounds.

Chromophore	Aldehyde[a]	Protonated Schiff-base[b]	Pigment[c]	Δ/cm^{-1}
All-*trans* (**1**)	382	440	560	4870
Naphthyl analogue (**2**)	368	425	504	3689

[a] In EtOH. [b] Protonated Schiff-base with 2-aminoethanol, in MeOH. [c] In buffer, pH 5·0.

All theoretical models to explain the colour of retinal-based pigments have been influenced by the original work of Pitt *et al.*,[9] who showed that protonated Schiff-bases of retinal are red-shifted to *ca.* 440 nm and that their spectra are sensitive to the nature of the solvent and the counter ion. It



Scheme 1. Reagents: i, Diethyl ethoxycarbonylmethylphosphonate/NaH (ref. 10), ii, $LiAlH_4$–$AlCl_3$, iii, MnO_2, iv, acetone, 1N NaOH.

has, therefore, been argued that a protein, through the placement of suitable groups around the retinylidinium chromophore, could regulate the spectra of rhodopsin. Approaches using the spectral properties of pigments derived from judiciously modified analogues of retinal have played an important role in the evaluation of the various theoretical models. In this communication we describe the synthesis of a conformationally rigid naphthyl analogue (**2**) of retinal and report on its interaction with bacterio-opsin.

The all-*trans*-naphthyl analogue [(**2**) m.p. 156—158 °C; λ_{max} 368 nm, ϵ 47,000] was prepared as indicated in Scheme 1 and shown to combine with bacterio-opsin, at a rate which was about 3 times faster than that of a similar reaction with all-*trans*-retinal, to furnish a new pigment absorbing at λ_{max} 504 nm (ϵ 43,000). That the analogue was bound to the site normally occupied by the natural chromophore was *indicated* by competitive experiments.† The formation of a Schiff-base linkage between the analogue and the protein was established by the $NaBH_4$ reduction of a suitable labelled species.‡

The efficacy of the interaction of the naphthyl analogue with bacteriorhodopsin may be assessed by using the criterion suggested by Nakanishi *et al.*[11] The authors have argued that the contribution which the microenvironment of an opsin makes to the spectrum of a pigment may be measured in terms of 'opsin shift' which is expressed as Δ (cm^{-1}) and is defined as: λ_{max} (in cm^{-1}) of a protonated Schiff-base (synthesised from the aldehyde and a primary amine) *minus* the λ_{max} (in cm^{-1}) of the pigment generated from the same aldehyde.

Using this criterion, Δ (cm^{-1}) for the retinal-derived bacteriorhodopsin is 4870 cm^{-1}, whereas for the artificial pigment obtained from the napthyl analogue, Δ = 3690 cm^{-1}. These results show that the 'opsin shift' for the artificial pigment is about 75% of that for bacteriorhodopsin, thus suggesting that in both cases, the chromophores experience similar stereoelectronic interactions at the retinal-binding site.

† Bacterio-opsin (17 nmol) was suspended in 200 mM potassium phosphate buffer, pH 5·0, in the presence of NaCl, and then incubated with the naphthyl analogue (18 nmol) for 60 min, when the formation of the artificial pigment (λ_{max} 504 nm) was complete. After this period, the incubation mixture was supplemented with all-*trans*-retinal (30 nmol). The amount of bacteriorhodopsin (λ_{max} 560 nm) which was formed in the second stage of the experiment was less than 10% that of the pigment absorbing at 504 nm.

‡ The [1-^{3}H]naphthyl analogue was prepared and used to form the [^{3}H]-pigment. The latter was treated with $NaBH_4$ and it was found that 1 mol of ^{3}H from the analogue was covalently bound to 1 mol of the protein.

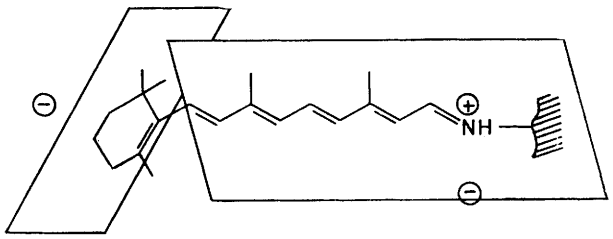


Figure 1

In the light of the observation recorded above, we may examine the most compelling theoretical model,[11,12] now in vogue, to account for the red-shift in bacteriorhodopsin. The model depicted in Figure 1 makes two broad assumptions. Firstly that in bacteriorhodopsin, the β-ionone ring with respect to the polyene chain is twisted around the C-6, C-7 bond by about 45°. The naphthyl analogue (**2**), employed in the present study may be regarded as representing all-*trans*-retinal in which carbons 8 and 18 are fixed by a ring, forcing the polyene chain and the ring to adopt a planar conformation. That such an analogue interacted favourably with bacteriorhodopsin suggests a view of the retinal-binding site in which the ring–chain conformation approaches planarity.[15]

The second assumption made in the model arises from the knowledge that the excitation of a Schiff-base results in the accumulation of negative charge in the vicinity of the nitrogen, leaving a positive charge in the polyene chain, which it was argued migrates towards the β-ionone ring. The presence of a negative point-charge located near the β-ionone ring as shown in Figure 1 would then stabilise the excited state and facilitate the red shift. Consequently it was predicted, albeit on intuitive grounds, that tailored retinals, with altered rings of the type which exists in the aromatic analogue (**3**), and therefore by implication in (**2**), should interact with bacterio-opsin anomalously.§ In our view the results obtained with the naphthyl analogue (**2**) do not bear out this prediction and therefore require revision of the model using modified assumptions regarding the ring–chain conformation and the location of the point-charge.

§ In support of this assertion, the authors describe the formation of a pigment (λ_{max} 480 nm) from the phenyl analogue (**3**) (ref. 13) and bacterio-opsin which showed a considerably diminished 'opsin shift' of about 1100 cm^{-1}. An independent work (ref. 14) published soon after reported the same pigment to have λ_{max} 510 nm thus casting doubt on the validity of the data used in support of the original assertion (ref. 13).

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