Table I. DC Conductivity of **1**

T, °C	state and treatment	conductivity, Ω^{-1} cm^{-1}
30	solid untreated (L state)	(1×10^{-11})-(2×10^{-12})
110	liquid crystalline before "electric stimulus" (M state)	4×10^{-8} [a]
110	liquid crystalline after "electric stimulus" (H state)	$(1.2 \pm 0.2) \times 10^{-3}$ [b]

[a] Probably due to ionic current; see ref 4c. [b] Six independent experiments.

Below T_{C_1}, the conductivity enhancement was much smaller, or absent, and was not reproducible. Above T_{C_2}, a similar enhancement was observed but was followed by rapid electrolysis leading to carbonification. Therefore, the liquid-crystalline state is necessary for production and retainment of the H state.

A possible mechanism of the conductivity enhancement is accumulation of a $V_{O6}^{\bullet+}$-type intermediate (probably together with I_2 and/or I), based on direct measurements of the electrode system at 535- and 550–560-nm absorption ascribable to $V^{\bullet+}Br^-$ and $V^{\bullet+}I^-$, respectively, in a highly polar medium. The amount of $V^{\bullet+}$ (30 V) was determined at 680 nm to be 0.13% (after 4 min), 0.71% (7 min), and 5.5% (30 min). A thin layer of the blue H-state solid cut out along the cathode surface was dissolved in carefully deoxygenated $CHCl_3$ and showed the typical $V^{\bullet+}$ absorption[8] at 605 nm ($A = 0.31$, amounting to 5% of **1** employed). However, the $V^{\bullet+}$ absorption very quickly disappeared ($\tau_{1/2}$, a few seconds) when *all* of the blue H-state solid was dissolved in carefully deoxygenated $CHCl_3$. These observations strongly suggest that a $V_{O6}^{\bullet+}$ (and also a I_2/I) gradient was formed and that back electron transfer[9] regenerating $V^{2+}\cdot(I^-)_2$ took place readily in a solution but slowly in a liquid-crystalline or solid state (Chart I).

A conclusion then may be drawn that electrons are conducted via the less strongly bound electron on $V^{\bullet+}$, present probably in the form of aggregates (from the observed ESR broadening).

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Retinal Analogues with Locked 6–7 Conformations Show That Bacteriorhodopsin Requires the 6-*s*-*Trans* Conformation of the Chromophore

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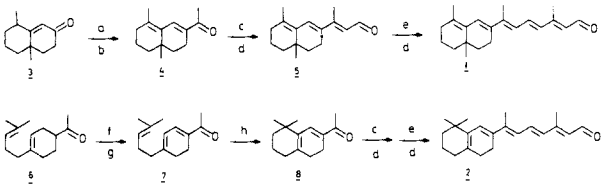
Bacteriorhodopsin (bR) functions as a light-driven proton pump in the purple membrane of *Halobacterium halobium*.[1,2] The chromophore is *all-trans*-retinal bound to the ϵ-amino group of lysine 216 via a protonated Schiff base (PSB) linkage.[3,4] It has recently been realized that the conformation about the C6–C7 single bond can play an important role in determining the spectroscopic properties of retinal-proteins.[5-7] In order to get more information how this conformation effects the properties of the chromophore both in vitro and in the protein, it is necessary to study bR analogues and model systems with a locked 6-*s*-*trans* and 6-*s*-*cis* chromophore. 8,16-Methanoretinal (**1**) is the retinal of choice in which the methylene group locks the polyene system in the 6-*s*-*trans* conformation with small or negligible changes in the electronic and steric factors. For comparison the interaction of a locked 6-*s*-*cis* analogue with bacterioopsin (bO) was also studied.

The synthesis of **1** and **2** is summarized in Scheme I. The starting bicyclic ketone **3** was prepared according to literature procedures.[8,9] Reaction of **3** with lithium acetylide at −60 °C and subsequent reaction with concentrated formic acid gives **4**,[10] which was extended by a Horner–Emmons type reaction[11] and Dibal reduction[12] to **5**. A Horner–Emmons reaction with the C_5-phosphonate and another Dibal reduction yielded retinal **1**. Retinal **2** was prepared starting from the Diels–Alder product of

Scheme I.[a] Synthesis of 8,16-Methanoretinal (**1**) and 8,18-Methanoretinal (**2**)



[a] (a) Acetylene/*n*-BuLi/THF. (b) HCOOH. (c) $(EtO)_2POCH_2CN$/NaH/THF. (d) Dibal. (e) $(EtO)_2POCH_2C(CH_3)$=CHCN/NaH/THF. (f) SO_2Cl_2/CCl_4. (g) DBN. (h) Concentrated H_2SO_4/toluene.

Table I. Absorption Maxima of Retinal Analogues, Their PSB's, and bR Analogues

analogue	retinal[a]	PSB[a]	bR	opsin shift,[b] cm^{-1}
all-trans-retinal	380 nm	440 nm	568 nm	5100
all-trans-**1**	400 nm	465 nm	564 nm	3800
all-trans-**2**	415 nm	485 nm	596 nm	3800

[a] Absorption maxima in methanol. [b] The opsin shift is the difference between the λ_{max} value of the protonated *n*-butylamine Schiff base of retinal analogue and that of the corresponding bR analogue.

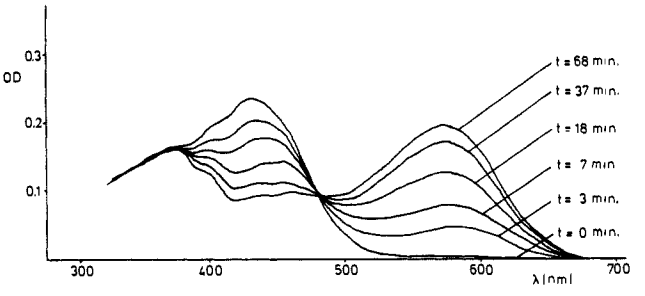


Figure 1. Formation of bR (**1**) analogue at 2 °C.

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myrcene and methyl vinyl ketone (**6**) (two possible isomers).[13] These isomers were first converted to the corresponding conjugated dienone **7** and its isomer. **7** could be purified by simple SiO_2 chromatography. **7** was converted with concentrated sulfuric acid into the locked β-ionone **8** in 91% yield.[14] **8** was converted into retinal **2** via the same four-step sequence as discussed for **1**. Both **1** and **2** were isolated in pure form by HPLC.[15]

In Table I the electronic data of retinals **1** and **2** and their PSB's are given. Retinal **1** reacts very rapidly with bO to form a bR analogue with λ_{max} 564 nm. It shows light–dark adaptation and has a proton pump efficiency of 90%.[16] To study the binding of **1** with bO more carefully, this reaction was carried out at 2 °C (Figure 1). First a 430–460-nm complex with vibrational fine structure is formed just as has been observed for retinal,[17] 5-demethylretinal,[18] and 7,8-dehydroretinal[19] and then the complex was fully converted to bR (**1**). This process is very similar to that of native bR. The rate of bR (**1**) formation is only 50% lower than that of bR.

The reaction of **2** with bO is much more complicated. An equimolar amount of **2** leads slowly to a pigment with λ_{max} 509 nm, close to λ_{max} of PSB (**2**). The spectrum shows a shoulder at 596 nm. Adding **2** in a very small amount, waiting for the binding to be complete, and adding further small amounts in similar fashion until the equimolar amount is reached lead to a two pigment mixture (λ_{max} 509 and 596 nm) in a 2:3 ratio. Similar complex behavior has been observed for 4-*n*-butyl- and 4-(dimethylamino)retinal.[20] We think that the λ_{max} 596 nm form is the fully regenerated bR (**2**) analogue, which shows a 3800 cm^{-1} opsin shift, whereas λ_{max} 509 nm form has a slightly larger λ_{max} value than the PSB. The pigment mixture does not show light–dark adaptation and has a 20% proton pump efficiency. These bioorganic studies indicate that a native bR structure can only be formed with the 6-*s-trans* conformer of retinal, in strong support of recent solid-state NMR studies.[5] Now that they are available, retinals **1** and **2** should be very important to establish the 6–7 conformation in rhodopsin and halorhodopsin.

Comparing the λ_{max} values of **1** (400 nm) and its PSB (465 nm) with those of retinal (380 and 440 nm, respectively) shows that twisted 6-*s-cis* → planar 6-*s-trans* isomerization results in a 1200–1300-cm^{-1} red-shift in the λ_{max} value. In the planar **1** and its derivatives, the 5–6 double bond is in full conjugation with the polyene chain; this is reflected in the larger λ_{max} value compared to retinal and its derivatives which are 40° twisted 6-*s-cis* conformers. In **2** the planar 6–7 bond is locked in the *s-cis* conformation giving rise to the expected 20-nm red-shift from the locked *s-trans* derivative.[21]

These data can now be used to understand the 5100-cm^{-1} opsin shift in bR.[22] Part of this shift, 1200 cm^{-1}, arises because the chromophore changes upon binding to the protein from a 40° twisted 6-*s-cis* conformation to a planar 6-*s-trans* conformation. For a retinal derivative that has a locked C6–C7 conformation, the observed opsin shift should be ~3800 cm^{-1} because no protein-induced change of the C6–C7 conformation is allowed, and that is what is observed for bR (**1**) and bR (**2**). Once the six-membered ring is fixed it does not contribute significantly to the opsin shift. This is consistent with the idea that the opsin shift is mainly due to the perturbation of the Schiff base region and this is in agreement with solid-state ^{15}N NMR[23] and retinal analogue[6,7] evidence that show that there is a weakened interaction of the Schiff base with the counterion in the protein.

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(15) Analytical data: exact mass calcd for **1** ($C_{21}H_{28}O$) 296.4516, found 296.4522. ^1H NMR (300 MHz, $CDCl_3$) of *all-trans*-**1**: δ 0.95 (1-CH_3, s), 1,80 (5-CH_3, s), 2.10 (9-CH_3, s), 2.34 (13-CH_3, s), 5.98 (H14, d, J = 8 Hz), 6.41 (H12, d, J = 15 Hz), 6.42 (H10, d, J = 11 Hz), 6.77 (H7, s), 7.20 (H11, dd, J = 15, 11 Hz), 10.11 (H15, d, J = 8 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) of *all-trans*-**1**: δ 13.2 (C19), 14.3 (C20), 18.5 (C3), 18.9 (C18), 23.0 (C16a), 23.3 (C17), 33.3 (C4), 33.8 (C1), 37.3 (C16), 37.7 (C2), 124.2 (C14), 124.4 (C10), 128.7 (C7), 132.2 (C5), 133.3 (C11), 134.3 (C8), 134.4 (C12), 135.1 (C6), 142.6 (C9), 155.0 (C13), 191.1 (C15). Exact mass calcd for **2** ($C_{21}H_{28}O$) 296.4516, found 296.4518. ^1H NMR (300 MHz, $CDCl_3$) of *all-trans*-**2**: δ 1.04 (1,1'-CH_3, s), 2.09 (9-CH_3, s), 2.34 (13-CH_3, s), 5.97 (H14, d, J = 8 Hz), 6.34 (H7, s), 6.38 (H12, d, J = 15 Hz), 6.39 (H10, d, J = 11 Hz), 7.20 (H11, dd, J = 15, 11 Hz), 10.10 (H15, d, J = 8 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) of *all-trans*-**2**: δ 13.2 (C19), 14.2 (C20), 19.3 (C3), 23.8 (C18a), 28.6 (C16/C17), 29.5 (C18), 31.1 (C4), 32.6 (C1), 39.1 (C2), 123.9 (C14), 124.3 (C10), 128.7 (C7), 132.6 (C5), 133.4 (C11), 134.1 (C12), 135.0 (C8), 135.3 (C6), 141.9 (C9), 155.1 (C13), 191.1 (C15).
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Application of the Savage–Wood Treatment to the Quantitative Analysis of Kinetic Solvent Effects in Highly Aqueous Binary Solutions

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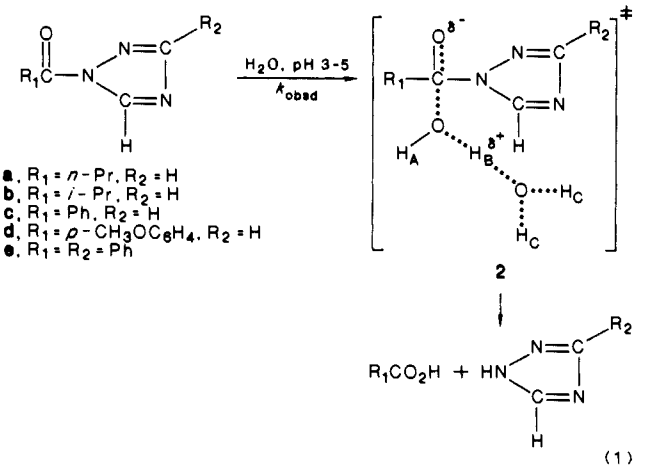
The interpretation of kinetic solvent effects on organic reactions in water-rich binary mixtures is notoriously difficult.[2] Herein we wish to present an attempt to analyze these medium effects quantitatively, using an extension of the Savage–Wood treatment of solute–solute interactions.[3] To this end, we have measured pseudo-first-order rate constants for the water-catalyzed hydrolysis of 1-acyl-1,2,4-triazoles (**1a–e**) in highly aqueous alcohol–water and 1,4-dioxane–water mixtures (eq 1). The reaction mechanism



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