deprotection of **10** affords **2a**, carba-D-fructofuranose, in excellent yield (Scheme I).[10] Phosphorylation of **10** with dibenzylchlorophosphate and complete deprotection provided **2b**, carba-D-fructose 6-phosphate.

The important result from biological testing of **2b** is that these carba analogues of fructose are indeed substrates for the relevant enzymes of the glycolytic pathway.[11] Analogue **2b** is an excellent substrate for phosphofructokinase and 6-phosphofructo-2-kinase, with K_m from 5–20 times larger than that of the natural substrate, Fru-6-P. The analogue inhibits fructose-2,6-bisphosphatase with K_i about 500 times higher than that of Fru-6-P. Finally, preliminary results reveal that the diphosphate generated from **2b** by the action of 2-kinase is a potent positive effector for phosphofructokinase.

This work demonstrates a practical synthetic approach to important new analogues of fructofuranoid enzyme regulators. The synthesis illustrates an interesting heuristic principle that can be used to plan future syntheses: the acyclic, unbranched, chiral, and elaborately functionalized carbon chains that are readily available from carbohydrates can be neatly converted to polyhydroxylated and branched carbocycles by the use of appropriate unitive synthons. Further applications of this concept are under investigation.

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(10) ^{1}H NMR (360 MHz, D_2O) δ 3.82 (1 H, dd, J = 8.7, 8.8 Hz) 3.75 (dd, 1 H, J = 5, 11 Hz), 3.67 (1 H, d, J = 8.7 Hz), 3.58 (dd, J = 7.3, 11 Hz), 3.51 (s, 2 H, 2.16 (dd, 1 H, J = 10, 14.6 Hz) 1.89 (m, 1 H) 1.45 (dd, J = 7.6, 14.6 Hz). ^{13}C NMR (90 Mz, D_2O) δ 81.32, 80.32, 79.24, 68.80, 66.43, 44.02, 36.21.

(11) Personal communication from Professor Kosaku Uyeda (University of Texas Health Science Center, Dalls), manuscript in preparation.

Mechanism for the Opsin Shift of Retinal's Absorption in Bacteriorhodopsin[1]

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Dihydro derivatives of retinal were first used by Nakanishi et al.[2] in their pioneering development of a point charge model to explain how bacteriorhodopsin (BR) shifts the absorption maximum of its retinal protonated Schiff base (PSB) chromophore to the red. They measured the protein-induced shift of the absorption maximum (the "opsin shift") for selectively saturated chromophores. The opsin shift was reported to drop from 4870 cm^{-1} in the native chromophore, to 2500 cm^{-1} in the 5,6-dihydro derivative, and to only 1000 cm^{-1} in the 7,8-dihydro derivative. These data indicated that a negative bacterio opsin charge located near $C_5=C_6$ of the β-ionone ring is responsible for the opsin shift. A variety of BR analogue experiments have been performed subsequently to test the point charge model.[3] In our own experiments[4] it was useful to reexamine the 5,6- and 7,8-dihydroretinal data. While our 5,6-dihydro spectra are in agreement with the earlier work, our measured opsin shift for the 7,8-dihydroretinal derivative (3500 cm^{-1}) is larger than the originally published value. A large opsin shift for the 7,8-dihydro derivative would not be expected to result from a single negative point charge perturbation located near $C_5=C_6$. Thus it is evident that the current picture for the mechanism of the opsin shift in BR must be revised. ^{13}C NMR experiments[5] have supported the initial proposal that there is a negative protein charge near $C_5=C_6$ and have suggested that

Table I. Absorption Maxima and Bacterio Opsin Shifts for Retinal Analogues

chromophore	native	5,6-dihydro ref 2	5,6-dihydro this work	7,8-dihydro ref 2	7,8-dihydro this work
aldehyde, nm[a]	381	370	368	338	340
protonated Schiff base, nm[b]	440, 465[d]	425	428	395	385
pigment, nm[c]	568	476	475	400	445
opsin shift, cm^{-1}	5100, 3900[d]	2500	2300	1000	3500

[a] Synthesis described in ref 8. [b] Chloride salt of the n-butylamine Schiff base in methanol. [c] The sample consisted of membrane sheets suspended in H_2O at 1.5 °C.[9] [d] These values are for a planar 6-s-trans PSB as the reference state for the opsin shift rather than the twisted 6-s-cis conformer found in solution.[10]

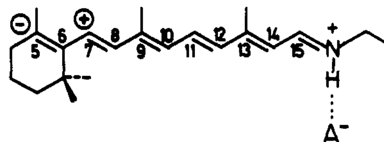

Figure 1. Model for the bacterio opsin binding site where the 6-s-trans chromophore interacts with a pair of opsin charges near $C_5 \cdots C_7$ The opsin shift data presented here support the idea that the retinal chromophore is perturbed by a negative charge near C_5 and a positive charge near C_7. However, it is also evident that the tendency for the negative charge near C_5 to red-shift the absorption is largely canceled out by the presence of the positive charge near C_7. This shows that much of the opsin shift is caused by protein–chromophore interactions near the Schiff base.

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there might be a positive opsin charge near C_7. The reduction of the opsin shift to 2300 cm^{-1} in 5,6-dihydro BR and its *increase* to 3500 cm^{-1} in 7,8-dihydro BR provides strong independent evidence for the positive charge near C_7. More importantly, the data presented here show that this positive charge is an important spectroscopic determinant and that protein perturbations near the Schiff base end of the chromophore play a dominant role in the production of the opsin shift. A similar reanalysis of the mechanism of the opsin shift has recently been developed independently by Nakanishi and co-workers.[6,7]

In Table I we present the absorption maxima of the protonated Schiff bases and of bacterio opsin regnerated with 5,6- and 7,8-dihydroretinal. The absorption maxima of native BR and its 5,6-dihydro derivative are in good agreement with the results of ref 2, while the λ_{max} of the 7,8-dihydro derivative is found to be 445 nm.[6] Thus, the opsin shift for 7,8-dihydro BR (3500 cm^{-1}) is much larger than that of the 5,6-dihydro derivative (2300 cm^{-1}). To compare these values with the opsin shift of the native chromophore it is necessary to separate electrostatic from ground-state conformational effects. ^{13}C NMR experiments[5] have shown that bacterio opsin preferentially binds the 6-s-trans conformer rather than the 30-70° twisted 6-s-cis conformer found in solution.[11] The absorption maximum for the s-trans conformer is calculated to be red-shifted by 16-32 nm from that of the twisted s-cis conformer,[12] and locked 6-s-trans PSB analogues are red-shifted by 25 nm.[10] By use of the experimental value, the opsin shift for the native chromophore can be partitioned into ~1200 cm^{-1} due to isomerization to a planar s-trans structure and ~3900 cm^{-1} due to other protein-chromophore interactions.

Figure 1 presents a model for the location of the protein charges which are important for the opsin shift in bacteriorhodopsin. A negative protein residue is placed near C_5 in agreement with the "point charge" model of Nakanishi et al.[2] In addition, a positive opsin charge is placed near the C_7 position of the chromophore. To understand how the opsin shift data lead to this model it is necessary to realize that in the excited state of the retinal PSB, positive charge shifts toward the ionone ring end of the chromophore, while in the ground state the positive charge is relatively localized near the Schiff base.[12-14] A negative environmental charge near the ionone ring would thus stabilize the energy of the excited state more than that of the ground state, leading to a red shift in the absorption. A positive environmental charge near the ionone ring would have the opposite effect.[15] In the 5,6-dihydro derivative the interaction of the conjugated chain with the negative charge is essentially eliminated and the opsin red shift falls to 2300 cm^{-1}. However, in this analogue a positive charge near C_7 would still interact effectively with the conjugated chain and should contribute to a reduction of the opsin shift. When the retinal chain is further shortened in the 7,8-dihydro derivative, we see the opsin shift *increase* to 3500 cm^{-1}. This rather dramatic biphasic behavior—that is, first a reduction in the opsin shift followed by an increase in the opsin shift as the chain is truncated—is a natural consequence of the interaction of the retinal chromophore with a pair of protein charges of opposite sign (or with a strongly dipolar residue) near the ionone ring. *These dihydro data thus provide strong evidence for a dipolar pair of opsin charges near the ionone ring in bacteriorhodopsin.*

In the 7,8-dihydro derivative, a large opsin shift of 3500 cm^{-1} remains. When this value is compared to the ~3900-cm^{-1} opsin shift for the native chromophore it is clear that *much of the bacterio opsin induced red shift must be due to the interaction of the protein with the Schiff base end of the chromophore.*[16] The red shift in BR is most easily explained by a weak hydrogen bond between the Schiff base proton and an electronegative group in the protein.[17] The chemical shift of ^{15}N-BR,[18] the perturbation of the $^{13}C_{15}$ resonance,[5] and recent resonance Raman data[19] strongly support this mechanism. These observations on the mechanism of the opsin shift in bacteriorhodopsin suggest that conformational distortions and the environment of the protonated Schiff base moiety may play a crucial role in λ_{max} regulation of other rhodopsins.

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Registry No. 5,6-Dihydroretinal, 19907-28-9; 7,8-dihydroretinal, 75917-44-1; retinal, 116-31-4.

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Multicoordinatively and Multielectronically Unsaturated Transition-Metal Complex Negative Ions: Generation and Chemistry of $(OC)_2Fe^{-\cdot}$ and $(OC)_3Mn^-$

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The presence of coordination unsaturation in organo-transition-metal complexes is required in many of the fascinating and useful reactions in organometallic chemistry, e.g., homogeneous catalysis,[1] many ligand substitution reactions,[2] CH activation,[3] and various synthetic organic transformations.[4] Studies of multicoordinatively and -electronically unsaturated (MCMEU)

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