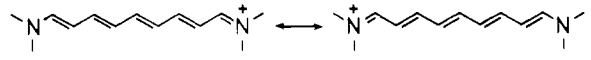
aldehyde **5b** yielded cyanine **2b** and merocyanine **1b**.[22]

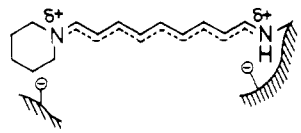
Purple membrane was isolated from *H. halobium* (strain R_1) according to Oesterhelt and Stoeckenius.[23] Addition of retinal analogues **1a** and **1b**, solubilized in less than 2% EtOH, to bleached purple membrane suspensions in 10 mM or 50 mM HEPES, pH 7.0 at 22 °C, in the dark, resulted in the formation of bR analogues with absorption maxima at 662 nm (Figure 2 and Table I). Due to the lability of the chromophores in buffer, it was necessary to use a ca. 5-fold molar excess of chromophores to attain maximum regeneration yields of the pigments. The pigments were reasonbly stable to 0.1 M NH_2OH at room temperature, i.e., there was less than 5% decrease of the 662-nm band in 5 h for the case of **1a** and in 2 h for the case of **1b**. The pigments thus formed were stable in the dark, only ca. 20% reduction in the 662-nm maxima being observed at 6 days at 22 °C. However, irradiation with light (>530 nm) at room temperature bleached 80% of the pigment from **1a** in 4 h and 90% of the methylated analogue within 40 min; shifts in the maxima of pigments, indicating formation of light-adapted species, could not be detected under these conditions.

It was not possible to apply the CH_2Cl_2 denaturation–extraction method[24] to check the integrity of the bound chromophore due to the instability of chromophores during the extraction procedure. The fact that chromophores **1a** and **1b** occupied the same binding site as *trans*-retinal was inferred from the following observations. The addition of *trans*-retinal to the maximally regenerated 662-nm pigments from **1a** and **1b** resulted in 38% and 52% growths, respectively, of the natural 560-nm peak. However, the 662-nm peak was not replaced by the 560-nm band, indicating that the retinal analogues were not displaced by *trans*-retinal. The biphasic nature of the CD curves (Table I) further supports occupation of the natural binding site.[25] The fact that these sites are not fully occupied by **1** is probably due to decomposition of the chromophore.[26] Further support that chromophores **1** were at the natural binding site was secured from the observation that no 662-nm pigment was formed upon addition of **1a** or **1b** to natural 560-nm bR (DA).

It is well established[27] that symmetric cyanines, exemplified by 9-(dimethylamino)nona-2,4,6,8-tetraenylidenedimethylammonium perchlorate **6**, λ_{max} (CH_2Cl_2) 625 nm (ϵ 295 000),[14]



6



7

have red-shifted absorption maxima characterized by narrow half-band widths ($W_{1/2}$) of ca. 1000 cm^{-1}. In contrast, in merocyanines having less uniform bond orders, the maxima are broad ($W_{1/2}$ ca. 6000 cm^{-1}) and blue-shifted. Note the broad and narrow bandwidths, respectively, of unsymmetric merocyanines **1** and symmetric cyanines **2** (Table I, entries i and iii). The fact that the two bacteriorhodopsins derived from **1** have *narrow bands absorbing at 662 nm* provided excellent evidence for the symmetric cyanine dye structure **7** of the chromophore in these pigments, i.e., *the data strongly support the external point-charge model as well as the SBH$^+$ linkage shown in Figure 1.* Theoretical calculations[28] fully corroborate these results. Preliminary experiments have also demonstrated that these cyanine pigments incorporated into vesicles lack the ability to translocate protons.

Acknowledgment. We are grateful to Drs. B. Honig, H. Kakitani, and T. Kakitani for discussions and to John D. Carriker for carrying out the binding studies. This work has been supported by NSF Grant CHE-8110505. C.G.C. was the recipient of an NIH postdoctoral fellowship award (F32GM07805).

Registry No. **1a**, 84215-22-5; **1b**, 84215-23-6; **2a**, 84215-25-8; **2b**, 84215-27-0; **3a**, 84215-28-1; **3b**, 84215-29-2; **4a**, 84215-30-5; **4b**, 84215-31-6; **5a**, 84215-32-7; **5b**, 84215-33-8; (*E*)-1-methoxy-1-buten-3-yne, 3685-20-9.

(22) Compounds **1b** and **2b** resisted crystallization. Merocyanine **1b** was purified by HPLC (Whatman Partisil ODS-2, 3% Et_3N in MeOH) immediately prior to binding experiments.

(23) Oesterhelt, D.; Stoeckenius, W. *Methods Enzymol.* **1974**, *31*, 667–678.

(24) Pilkiewicz, F. G.; Pettei, M. J.; Yudd, A. P.; Nakanishi, K. *Exp. Eye Res.* **1977**, *24*, 421–423.

(25) In the case of purple membrane, the positive and negative CD bands (bR^{DA} 525(+)/595(−) and bR^{LA} 535(+)/602(−)) were interpreted as a result of exciton interaction between bacteriorhodopsin molecules within the trimers that form the rigid, hexagonal lattice of the membrane: Heyn, M. P.; Bauer, P. J.; Dencher, N. A. *Biochem. Biophys. Res. Commun.* **1975**, *67*, 897–903. Becher, B.; Cassim, J. Y. *Biophys. J.* **1976**, *16*, 1183–1200. Ebrey, T. G.; Becher, B.; Mao, B.; Kilbride, P. *J. Mol. Biol.* **1977**, *112*, 377–397.

(26) The 480-nm chromophore band diminishes with binding (Figure 2). However, this is accompanied by growth of maxima at 425 and 315 nm, most likely arising from decomposition of the chromophore during binding.

(27) Griffiths, J. "Colour and Constitution of Organic Molecules"; Academic Press: New York, 1976; p 244.

(28) Following paper in this issue.

Symmetric Charge Distribution in the Bacteriorhodopsin Binding Site

Toshiaki Kakitani,† Hiroko Kakitani,† and Barry Honig*

Department of Biochemistry, Columbia University 630 West 168th Street, New York, New York 10032

Koji Nakanishi

Department of Chemistry, Columbia University New York, New York 10027

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In the preceding paper[1] the synthesis of two merocyanine analogues of retinal was described. The binding of these compounds to bacteriorhodopsin results in the formation of stable pigments having narrow absorption maxima above 640 nm with half-bandwidths of approximately 1200 cm^{-1}. These observations were interpreted in terms of the formation of a cyanine dye within the bacteriorhodopsin binding site. In this communication we present theoretical calculations that strongly suggest that the protein-bound cyanine dye interacts with a symmetric distribution of electric charge. Consequently our results lend strong support for the "external point-charge" model for bacteriorhodopsin published previously.[2]

The red-shifted absorption maxima and narrow bandwidths (1000 cm^{-1}) of symmetric cyanine dyes result from their highly delocalized π electrons. In constrast, hydrocarbon polyenes, which have only limited π-electron delocalization (and hence significant bond alternation), exhibit broad absorption bands (6000 cm^{-1}) and short-wavelength absorption maxima. The width of electronic absorption bands is dependent upon the displacement of the equilibrium nuclear configuration in going from the ground to the excited state. Nonzero displacements lead to the appearance of symmetric vibrational progressions in the absorption spectrum (the Franck–Condon effect) and hence to a broadening of absorption bands. As the displacement increases, a longer progression is observed, and the band is increasingly broadened. Since in linear conjugated systems increased delocalization and hence reduced bond alternation is associated with reduced geometry changes upon excitation, symmetric cyanines have narrow bands while hydrocarbon polyenes have broad bands.[3] Any perturbation that

† Present address: Department of Physics, Nagoya University, Chikusa-ku, Nagoya, 464, Japan.

(1) Derguini, F.; Caldwell, C.G.; Motto, M. G.; Balogh-Nair, V.; Nakanishi, K., preceding paper in this issue.

(2) Nakanishi, K.; Balogh-Nair, V.; Arnaboldi, M.; Tsujimoto, K.; Honig, B. *J. Am. Chem. Soc.* **1980**, *102*, 7945.

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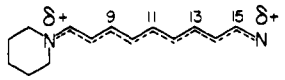
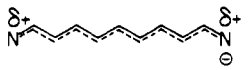
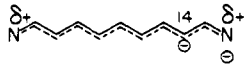
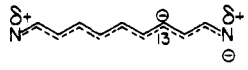
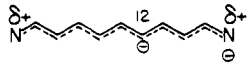
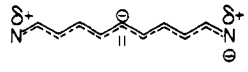
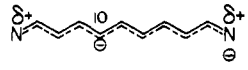
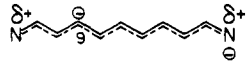
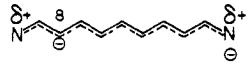
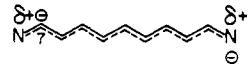
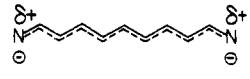
Table I. Calculated[a] Absorption Maxima and Half-Bandwidths[b] of a Cyanine Dye in the Field of External Charges

	λ_{max}, nm	R	$W_{1/2}$, cm^{-1}
	624	0.021	1000
	548	0.068	3500
	380	0.088	4500
	442	0.083	4000
	405	0.078	4000
	563	0.068	3500
	486	0.052	2500
	657	0.049	2500
	575	0.031	1500
	653	0.027	1500
	624	0.022	1000
	641	0.020	1000

[a] See ref 4 for a description of the calculations. [b] Estimated from $W_{1/2} = 1000 + 500(R - 0.02) \times 10^2$.

reduces the symmetry of a cyanine and increases bond alternation would thus be expected to blue-shift and broaden its electronic spectrum.

A model for the spectral shifts induced in the retinal chromophore by the bacteriorhodopsin binding site has been derived previously.[2] A counterion is placed approximately 3 Å from the protonated Schiff base linkage while a second negative charge is placed 3.5 Å above C-5. The two charges provide a symmetric perturbation and should have little effect on the delocalized π system of the cyanine chromophore. In contrast, other charge distributions might be expected to lower the extent of delocalization and increase bond alternation. It is important to determine whether these expectations are supported by theoretical calculations.

To this end we have carried out π-electron calculations on the cyanine dye interacting with point charges located in different positions around the chromophore. In Table I we report values of λ_{max} for a number of possible representations of the charge distribution in the bacteriorhodopsin binding site. The π-electron calculations were carried out with parameters that have been previously shown to yield excellent agreement with experiment for cyanine spectra.[4] The first entry in the table is for a cyanine dye without external charges, which corresponds to the isolated molecule in the absence of the protein. Agreement with experiment is excellent; the calculated and experimental[1,5] values for λ_{max} are both about 620 nm. For our calculations with external point charges we have placed a fixed charge 3 Å from the Schiff base nitrogen to represent the counterion[6] and have included a second charge positioned 3 Å away from different atoms in the system. From Table I it is evident that when two charges are used, satisfactory agreement with the experimental λ_{max} of about 650 nm is obtained only when the "mobile" charge is placed near atoms 6, 7, 8, or 10 in the chain. Although the exact position of the second charge is impossible to determine from these calculations, it must clearly be located in the ring half of the chain.

A further constraint on the position of the charge may be obtained from the narrow bandwidths of the cyanine dye pigments. McCoy and Ross[7] showed that the parameter $R = (\sum \Delta r_i^2)^{1/2}$ (where Δr_1 is the change in the ith bond length upon excitation) is a sensitive measure of the bandwidths of aromatic molecules. More recently, it was shown that the calculated values for R successfully account for the bandwidths of polyenes, cyanines, visual pigments, and bacteriorhodopsin.[3,4] The observed narrowing of the absorption bands of visual pigments as a function of λ_{max} was explained with this parameter, and it was demonstrated that these complex systems behaved spectroscopically as typical polyenes.[3,4] Wavelength-dependent absorption band shapes (nomograms) were deduced on this basis,[8] and these have found widespread application in visual pigment research.

In Table I we report values for R for each of the representations of the bacteriorhodopsin binding site. It is necessary to relate R to experimental bandwidths in order to determine which charge distributions are consistent with the 1200-cm^{-1} bandwidth of the cyanine dye pigment. For the isolated polyenes in solution, R was found to vary from about 0.02 Å for a cyanine dye to about 0.10 Å for a typical hydrocarbon polyene.[3] The bandwidth increases from 1000 cm^{-1} (cyanines) to 6000 cm^{-1} (polyenes) over this range of R's. As pointed out in the previous paragraph, the parameter R can account for the bandwidths of both visual pigments and bacteriorhodopsin, which have R values and bandwidths intermediate between the two extremes. As an example, the R value of bacteriorhodopsin is calculated, by using the external point charge model, to be 0.067 Å, and its bandwidth is approximately 3500 cm^{-1}. A straight-line fit to the three points defined by the model cyanine, polyene, and bacteriorhodopsin has a slope of approximately 650 cm^{-1} per 0.01 Å. Since the slope appears to increase somewhat for larger values of R, we choose a value of 500 cm^{-1} to approximate the bandwidths for the systems depicted in Table I.

It is clear from the table that the experimental bandwidth is reproduced only when the external charge is placed near atoms 6, 7, or 8 in the chain. This is the same set of atoms consistent with the constraint provided by λ_{max} (see above), with the exception of atom 10, which produces an unacceptably broad absorption band. Thus, the dual requirements of a 640-nm band and a width of 1200 cm^{-1} can only be satisfied with an external charge located quite close to the ring end of the polyene chain.

The experimental data obtained with the cyanine analogues of bacteriorhodopsin[1] together with the theoretical calculations (Table I) provide strong evidence that the chromophore interacts with a symmetric distribution of charges. This is in complete agreement with the external point-charge model for bR. The external point-charge model for bovine rhodospin places the second negative charge near carbons C_{12}–C_{14}.[9] From Table I we would predict that if a cyanine were bound to the apoprotein, the resulting

(3) Greenburg, A.; Honig, B.; Ebrey, T. G. *Nature (London)* **1975**, *257*, 823.
(4) Honig, B.; Greenberg, A.; Dinur, U.; Ebrey, T. G. *Biochemistry* **1976**, *15*, 4593.
(5) Malhotra, S.; Whitting, M. *J. Chem. Soc.* **1960**, 3812.
(6) The location for the counterion depicted in Table I is schematic and not intended to locate its position precisely. Somewhat different positions based on conformational energy calculations have been suggested (R. Birge, private communication), but these in no way affect the conclusions of this work.
(7) McCoy, E. F.; Ross, F. G. *Aust. J. Chem.* **1962**, *15*, 573.
(8) Ebrey, T. G.; Honig, B. *Vision Res.* **1977**, *17*, 147.
(9) Honig, B.; Dinur, U.; Nakanishi, K.; Balogh-Nair, V.; Gawinowicz, M.; Arnaboldi, M.; Motto, M. G. *J. Am. Chem. Soc.* **1979**, *101*, 7084.

visual pigment would have a broad ($W_{1/2}$ ca. 4000 cm^{-1}) blue-shifted absorption band. Attempts to form cyanine analogues of bovine rhodopsin are currently underway.

Acknowledgment. We are grateful to Hillary Rodman for her assistance in carrying out the calculations and to Dr. Valeria Balogh-Nair for discussions. This work has been supported by NSF Grants PCM82-07145 and GM-30519 (to B.H.) and NSF Grant CHE-8110505 (to K.N.).

Registry No. Retinal, 116-31-4.

Wittig Olefination via Reaction of Fluorine-Containing Phosphoranium Salts and *F*-Acyl Fluorides. A New Approach to Fluoroolefin Synthesis[1]

Donald J. Burton* and Daryl G. Cox

Department of Chemistry, University of Iowa
Iowa City, Iowa 52242

Received September 29, 1982

The normal course of reaction between phosphonium ylides and acyl halides is acylation of the ylide. If the new acylated salt contains a hydrogen on the carbon atom α to the phosphonium salt center, further acid–base reaction (transylidation) occurs to afford a new ylide:

$$\underset{\text{I}}{R_3P^+-{}^-CHR^1} + R^2C(O)Cl \rightarrow [R_3P^+-CHR^1C(O)R^2]Cl^- \xrightarrow{\text{I}} [R_3P^+-{}^-CR^1C(O)R^2]$$

We report the first preparation of a fluorine-containing phosphoranium salt[2] via reaction of fluorotrihalomethanes and tertiary phosphines:[3]

$$\underset{\text{II}}{3\ R_3P:} + \underset{\text{III}}{CFX_3} \rightarrow \underset{\text{IV, 90–95\%}}{[R_3P^+-{}^-CF-{}^+PR_3]X^-} + R_3PX_2$$

R = Bu, Ph; X = Cl, Br

When R = Bu, salt IV[4] readily undergoes a Wittig reaction with *F*-acyl fluorides (V) to give *F*-vinylphosphonium salts (VII) (only the *Z* isomer is observed). Subsequent hydrolysis of the vinylphosphonium salt provides a stereospecific route to the chain-extended (*E*)-1-hydro-*F*-olefin[5] VIII (Scheme I). The use of V introduces a stronger carbon–halogen bond into VI and retards elimination of halide ion (which leads to the acylation product).[6] In addition, the use of IV assures that the charged oxygen atom in VI must occupy a gauche position with respect to one of the two phosphorus atoms. Thus, this system permits one to compare the resultant rate of ring closure of VI to loss of halide ion and to determine whether Wittig olefination can compete with the acylation route.

When R = Bu(alkyl) in IV, reaction with V is rapid and VII is formed in 70–82% yields (as determined by ^{19}F NMR analysis). Little or none of the acylated product is observed.[7] In addition,

Scheme I

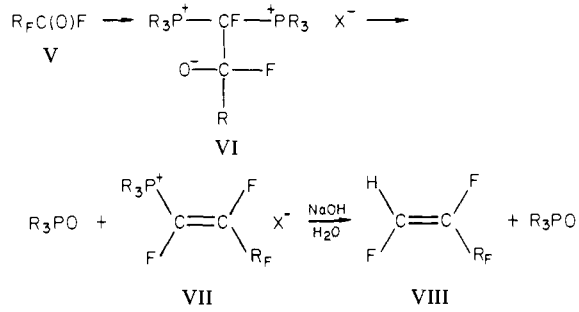


Table I

$$IV^a + V \rightarrow VII \xrightarrow[H_2O]{NaOH} VIII$$

R_F	VII,[b] %	VIII,[c,d] %
CF_3	73	45
CF_3CF_2	80	62
$CF_3CF_2CF_2$	82	52
CF_2Cl	73	50
$CH_3O_2CCF_2$	70	20
$CF_3(CF_2)_2OCF(CF_3)$	75	49

[a] The phosphoranium salt was generated in all but the last case from 0.150 mol of Bu_3P and 0.050 mol of $CFCl_3$. In the $CF_3(CF_2)_2OCF(CF_3)COF$ case, $CFBr_3$ was utilized. [b] ^{19}F NMR yield vs. C_6F_6. [c] Isolated yield of pure olefin. [d] The NMR, MS, and IR data were fully consistent with the assigned structures.

only one isomer of VII is detected by ^{19}F NMR—the *Z* isomer as noted in Scheme I. None of the *E* isomer was detected within the limits of ^{19}F NMR analysis. Table I summarizes the data for representative examples of V. Halogen, ether, and ester functionality is tolerated without any difficulty.

Stereospecific hydrolysis of VII occurs readily with addition of 50% NaOH, and the resultant chain-extended fluoroolefin is obtained in modest isolated yields. Table I illustrates typical examples. Since the preparation of IV, reaction with V, and the hydrolysis of VII can be carried out in a one-pot sequence, this novel approach to the synthesis of fluoroolefins from acyl fluorides provides a convenient synthetic entry to these versatile materials.

In contrast to the facile reaction of IV and V to give VII,[8] when the corresponding *F*-acyl chloride $R_FC(O)Cl$ is utilized in this reaction sequence (Scheme I), VII is *not* detected in any appreciable amount. Only acylation and cleavage products of IV were observed. Thus, the effect of the introduction of the stronger carbon–fluorine bond in VI is dramatically illustrated.

Operational details of the experimental procedure are outlined below for the preparation of (*E*)-1,2,3,3,4,4,5,5,5-nonafluoro-1-pentene.

A 250-mL three-necked flask, equipped with magnetic stir bar, rubber septum, and nitrogen tee, was charged with 0.150 mol (30.3 g, 37.4 mL) of tri-*n*-butylphosphine and 60 mL of dry benzonitrile.[9] The solution was cooled in an ice bath, and 0.050 mol (6.9 g, 4.7 mL) of trichlorofluoromethane was added in one portion via syringe. The resultant reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 3 h. ^{19}F NMR analysis indicated a 95% yield of $[Bu_3{}^+PC^-FP^+Bu_3]Cl^-$. To this phosphoranium salt solution was added ~0.050 mol of *F*-butanoyl fluoride [prepared from 0.055 mol (11.8 g, 7.2 mL) of *F*-butanoic acid]. Rapid reaction occurred to give 85% (*Z*)-$[Bu_3{}^+PCF{=}CFC_3F_7]X^-$. Addition of 6 mL of 50% NaOH, followed by flash distillation, drying over anhydrous $MgSO_4$, and fractional distillation gave 6.0 g (bp 32–34 °C) of >95% pure (*E*)-

(1) Presented in part at the 10th International Symposium of Fluorine Chemistry, Vancouver, Canada, Aug 1982, Abstract O-5.

(2) Cf. Ramirez et al. (Ramirez, F.; Pilot, J. F.; Desai, N. B.; Smith, C. P.; Hansen, B.; McKelvie, N. *J. Am. Chem. Soc.* **1967**, *89*, 6273) for the use of the term phosphoranium salt.

(3) To our knowledge these fluorine-containing phosphoranium salts are the first examples of fluoromethylene ylides that have been detectable.

(4) When R = Ph, the phosphoranium salts are inert to V.

(5) Cf. Burton et al. (Burton, D. J.; Inouye, Y.; Headley, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 3980) for a previous report of chain-extention reactions as routes to fluoroolefins and fluorodienes.

(6) The use of acyl fluorides in place of acyl chlorides with simple ylides such as $Ph_3{}^+PC^-(Me)_2$ is not sufficient by itself to divert the overall process from acylation to olefination. It's the combination of effects in the process outlined in Scheme I that results in overall Wittig olefination.

(7) When a nonfluorinated acyl fluoride is utilized, olefination is not observed—only acylation results.

(8) In contrast to the facile reactions observed with IV, $[Bu_3{}^+PC^-ClP^+Bu_3]Cl^-$ did not react with $CF_3CF_2CF_2COF$.

(9) Solvents such as methylene chloride, chlorobenzene, dioxane, and *o*-chlorotoluene can also be utilized to prepare IV. Best results in subsequent reactions of IV were obtained with benzonitrile and *o*-chlorotoluene.

0002-7863/83/1505-0650$01.50/0