(8, 80%), which in THF is in equilibrium with 7 and ethylene. The *t*-Bu substituents evidently provide a more rigid cavity, capable of protecting sensitive ligands. In THF, 7 reacts with CO to give green paramagnetic $[Ni(NS_3{}^{tBu})CO]^+$ (9, 60%, ν_{CO} 2026 cm^{-1}; g = 2.005, 2.114, Ni-C 1.81 (1) Å, Ni-S 2.36-2.37 Å),[7] in which the Ni-N bond (2.22 (1) Å) trans to the carbonyl (Ni-C-O 178 (1)°) is longer than that in other members of the set.

This work provides the initial demonstrations of stable, structurally defined [Ni^{II}-Me], [Ni^{II}-COMe], [Ni^{II}-H], and [Ni^{I}-CO] species and the reaction sequence [Ni-Cl] → [Ni-CH_3] → [Ni-$COCH_3$] → CH_3COSR',[12] all in the absence of tertiary phosphine/arsine and (other) carbon ligands. While [Ni-$(NS_3{}^R)$]-type species cannot at present be claimed as analogues of different states of the Ni site in CODH, the existence and reactivity of such species lend viability to current views of certain steps in the operation of *C. thermoaceticum* CODH.[1d,3,9,13] Future accounts will provide further details on the reactivity of species based on the [$Ni(NS_3{}^R)$] coordination unit.

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Supplementary Material Available: Atom positional parameters for the BPh_4^- salts of **1-4**, **7**, and **9** (7 pages). Ordering information is given on any current masthead page.

(12) The identity of the product Ni complexes is under investigation.
(13) Harder, S. R.; Lu, W.-P.; Feinberg, B. A.; Ragsdale, S. W. *Biochemistry* **1989**, *28*, 9080.

Bovine Rhodopsin with 11-Cis-Locked Retinal Chromophore neither Activates Rhodopsin Kinase nor Undergoes Conformational Change upon Irradiation

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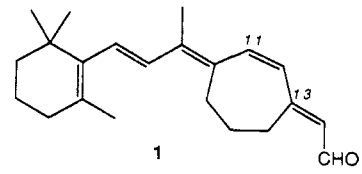
Rhodopsins belong to the multigene family of receptors which share the structural feature of seven membrane-spanning helices.[1] While most of these proteins are activated by soluble ligands, rhodopsins are activated by covalently bound 11-*cis*-retinal, It has been proposed that rhodopsin photoactivation results from 11-cis to all-trans isomerization of retinal;[2] an intermediate in the relaxation process of the conformationally excited pigment, metarhodopsin II (meta II), interacts with a series of soluble proteins, terminating in cGMP hydrolysis on one hand[3] and in phosphorylative deactivation of rhodopsin by rhodopsin kinase (RK) on the other.[4]

The 11-cis-locked cycloheptenediylidene analogue **1** forms nonbleachable pigments with opsins from various sources;[5,6] however, **1** and its 13-cis isomer, upon addition to bleached salamander rod outer segments, restores some light sensitivity through a mechanism apparently unrelated to the photoexcitation of the pigment.[7] Indirect studies with the green alga *Chlamydomonas reinhardtii* indicate that **1** restores phototaxis to a mutant that lacks retinal, also by an unknown mechanism.[8] Irradiation of bovine rhodopsin containing analgoue **1** (**1**-rhodopsin) forms two unstable intermediates as observed by picosecond measurements, a 580-nm species that is further excited to a 630-nm species,[9-11] which decays by fluorescence. In an effort to further clarify which properties of retinal are necessary and sufficient for transduction, **1**-rhodopsin was tested for (i) phosphorylation by preparations of rhodopsin kinase and (ii) conformational changes by difference FTIR spectroscopy.



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Rhodopsin Kinase (RK) Assays. A solution of RK was prepared by established methods.[12,13] Retinal-free opsin was prepared by hexane washing with slight modifications of standard methods.[14,15] Pigments were then prepared by incorporation of chromophore into opsin, 10 mM HEPES (pH 7), and further hexane washing of pigments to remove excess chromophore. RK assays were performed as previously described with some modifications.[16] Hexane-washed opsin and **1**-rhodopsin showed no light-dependent phosphorylation (Figure 1, curves 1 and 2), while 11-cis-regenerated rhodopsin gave reasonable activity (curve 3). Phosphorylation stoichiometry of hexane-washed pigments, with a maximum of 0.15 phosphates added/pigment molecule, was generally low. Treatment of opsin with 11-*cis*-retinal, *but with no further hexane wash*, restored highest activity (curve 4); the higher activity of this pigment indicates that hexane washing decreases transduction ability and may account for the low incorporation observed. The kinase assays thus show that **1**-rhodopsin from bovine opsin has very little, if any, light-dependent biochemical activity.[17]

FTIR Difference Spectra of Rhodopsin/Metarhodopsin II In-

(1) Hall, Z. W. *Trends Neurosci.* **1987**, *10*, 99-101.
(2) Hubbard, R.; Kropf, A. *Proc. Natl. Acad. Sci. U.S.A.* **1958**, *44*, 130-139.
(3) (a) Stryer, L. *Annu. Rev. Neurosci.* **1986**, *9*, 87-119. (b) Bennett, N.; Michel-Villez, M.; Kuhn, H. *Eur. J. Biochem.* **1982**, *127*, 97-103.
(4) Kuhn, H.; Cook, J. H.; Dreyer, W. J. *Biochemistry* **1973**, *12*, 2495-2502.
(5) Akita, H.; Tanis, S. P.; Adams, M.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1980**, *102*, 6370-6372.
(6) Crouch, R.; Nodes, B. R.; Perlman, J. I.; Pepperberg, D. R.; Akita, H.; Nakanishi, K. *Invest. Ophthalmol. Visual Sci.* **1984**, *25*, 419-428.
(7) Corson, D. W.; Cornwall, M. C.; MacNichol, E. F.; Jin, J.; Johnson, R.; Derguini, F.; Crouch, R. K.; Nakanishi, K. *Proc. Natl. Acad. Sci. U.S.A.*, in press.
(8) (a) Foster, K. W.; Saranak, J.; Derguini, F.; Rao, V. J.; Zarrilli, G. R.; Okabe, M.; Fang, J.-M.; Shimizu, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1988**, *110*, 6588-6589. (b) Foster, K. W.; Saranak, J.; Derguini, F.; Zarrilli, G. R.; Okabe, M.; Nakanishi, K. *Biochemistry* **1989**, *28*, 819-824. (c) Nakanishi, K.; Derguini, F.; Rao, V. J.; Zarrilli, G.; Okabe, M.; Lien, T.; Johnson, R.; Foster, K.; Saranak, J. *Pure Appl. Chem.* **1989**, *61*, 361-364.
(9) Buchert, J.; Stenfacic, V.; Doukas, A. G.; Alfano, R. R.; Callender, R. H.; Pande, M. J.; Balogh-Nair, V.; Nakanishi, K. *Biophys. J.* **1983**, *43*, 279-283.
(10) Birge, R. R.; Murray, L. P.; Pierce, B. M.; Akita, H.; Balogh-Nair, V.; Findsen, L. A.; Nakanishi, K. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4117-4121.
(11) Kandori, H.; Matsuoka, S.; Shichida, Y.; Yoshizawa, T.; Ito, M.; Tsukida, K.; Balogh-Nair, V.; Nakanshi, K. *Biochemistry* **1987**, *28*, 6460-6467.
(12) Palczewski, K.; McDowell, J. H.; Hargrave, P. A. *J. Biol. Chem.* **1988**, *28*, 14067-14073.
(13) Rando, R. R.; Seckler, B., unpublished.
(14) Papermaster, D.; Dreyer, H. *Biochemistry* **1974**, *13*, 2438.
(15) Fukada, Y.; Shichida, Y.; Yoshizawa, T.; Ito, M.; Kodama, A.; Tsukida, K. *Biochemistry* **1984**, *23*, 5826-5832.
(16) To each well of a 96-well culture plate were added a glass bead, RK solution (75 μL), nucleotide solution (20 μL, 7 mM ATP, 8 mCi [^{32}P]ATP in 100 mM Na_2PO_4 (pH 7.0), 5 mM $MgCl_2$), and pigment solution (50 mL). The wells were photolyzed at different intensities for 30 s with 480-510-nm light and shaken at 100 rpm for 20 min in the dark. The reaction was quenched by transfer of 120 μL from each well to a row of wells in a dot blotter containing 500 μL of 30% CCl_3COOH, filtering through Whatman GF/B filters. The blot was placed in a bath of cold 7 mM ATP in phosphate buffer and shaken gently for 1 h, and the spots were cut out and counted.

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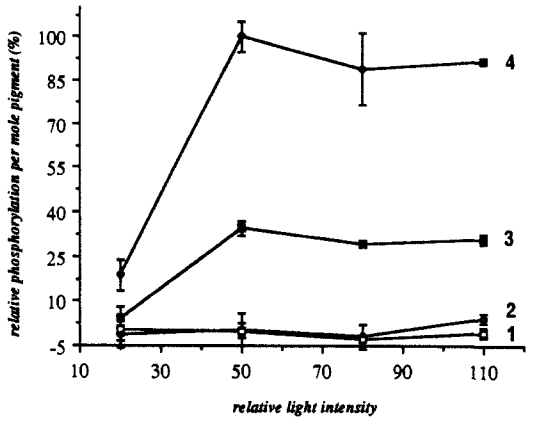

Figure 1. Rhodopsin kinase assay of hexane-washed bovine opsin and pigments. Curve 1: **1**-rhodopsin (hexane washed). Curve 2: Opsin (hexane washed). Curve 3: Rhodopsin (hexane washed). Curve 4: Retinal-free opsin treated with 11-*cis*-retinal (no further hexane wash).

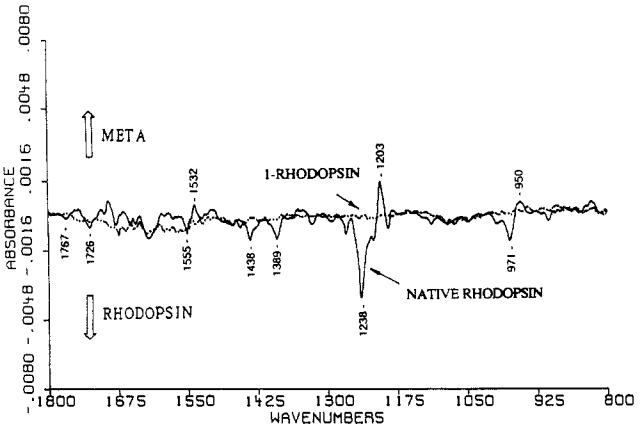

Figure 2. Solid curve: Difference FTIR spectrum between metarhodopsin II (shown as positive peaks) and rhodopsin (shown as negative peaks) in rhodopsin regenerated from hexane-washed bovine opsin and 11-*cis*-retinal. Dotted curve: Difference FTIR spectrum between metarhodopsin II and rhodopsin in **1**-rhodopsin regenerated from hexane-washed bovine opsin and retinal analogue **1**.

termediate Regenerated from Native 11-*cis*-Retinal and 11-Cis-Locked 1. The difference spectra (Nicolet 7199, 256 scans with full aperture, mirror velocity 45 cm/s, resolution 2 cm^{-1} at room temperature) were measured with films deposited on a ZnS window with samples prepared as described earlier.[18] The meta II stage was obtained by 250-W irradiation through a 500-nm filter, 1 min, 24 °C, its formation being checked by characteristic changes in carboxylic acid peaks in the difference FTIR spectra,[19] and visible absorption spectra of hydrated films on quartz. The difference spectrum of *native* red outer segment and meta II matched published data,[19] but that of pigment regenerated from 11-*cis*-retinal showed additional peaks at 1203 and 950 cm^{-1} arising from the meta I intermediate[20] (Figure 2); the difference visible spectrum also showed the presence of meta I as a 490-nm shoulder on the main 380-nm meta II peak. No change is seen in the difference spectrum between **1**-rhodopsin and the corresponding "meta II" (Figure 2). Thus, despite the sensitivity of the FTIR difference technique which can detect single protonation/deprotonation changes,[18,19] **1**-rhodopsin shows changes in neither the protein nor the chromophore moieties.

The RK and FTIR results corroborate the lack of light dependency seen in the partial restoration of sensitivity by analogue **1** in the bleached salamander rods. However, these results do not parallel those in the *Chlamydomonas* assay, in which **1** restored phototactic ability in a light-dependent manner.[8] Studies on the bovine, salamander, and *Chlamydomonas* systems are ongoing,

Acknowledgment. We are grateful to Professor Bob Rando for discussions and suggestions. The work has been supported in part by NIH Grant GM 36564.

(17) Phosphodiesterase assays on the analogue pigments indicated some activity which was less than that observed for rhodopsin. However, inability to establish an opsin blank for these experiments prevents us from making any conclusions.

(18) Bagley, K. A.; Balogh-Nair, V.; Croteau, A. A.; Dollinger, G.; Ebrey, T. G.; Eisenstein, L.; Hong, M. K.; Nakanishi, K.; Vittitow, J. *Biochemistry* **1985**, *24*, 6055.

(19) (a) Siebert, F.; Mantele, W.; Gerwert, K. *Eur. J. Biochem.* **1983**, *136*, 119–127. Rothschild, K.; Cantore, W. A.; Marrero, H. *Science* **1983**, 1333–1334. (b) de Grip, W.; Gray, D.; Gillespie, J.; Bovee, P. H. M.; van den Berg, E. M. M.; Lugtenberg, J.; Rothschild, K. *Photochem. Photobiol.* **1988**, *48*, 497–504.

(20) Ganter, U. M.; Kräutle, R.; Rando, R. R.; Siebert, F. In *Molecular Physicology of Retinal Proteins*; Hara, T., Ed.; Yamada Science Foundation: Osaka, 1988; pp 55–60.

Additions and Corrections

Electrically Conductive Metallomacrocyclic Assemblies. High-Resolution Solid-State NMR Spectroscopy as a Probe of Local Architecture and Electronic Structure in Phthalocyanine Molecular and Macromolecular "Metals" [*J. Am. Chem. Soc.* **1986**, *108*, 437–444]. PAUL J. TOSCANO and TOBIN J. MARKS*

Page 443: The negative sign in eq 1 should be moved from the rightmost to the middle term and the signs of the a_i values in Table II adjusted appropriately. This typographical error in no way affects the key conclusions of this work.

Experimental and Theoretical Evidence for Nonlinear Coordination of "sp-Hybridized" Carbon Atoms: The Gas-Phase Structure of Trifluoroethylidynesulfur Trifluoride, CF_3—C≡SF_3 [*J. Am. Chem. Soc.* **1987**, *109*, 4009–4018]. DINES CHRISTEN, HANS-GEORG MACK, COLIN J. MARSDEN,* HEINZ OBERHAMMER,* GABRIELE SCHATTE, KONRAD SEPPELT, and HELGE WILLNER

The bending angles [θ = 180 − CCS] given in the theoretical calculations for CF_3—C≡SF_3 in Table VIII and in Figure 6 (p 4016) are too large by a factor of 2. Similarly, the calculated energy minima in the Note Added in Proof should read as follows: 180° (HF), 162° (MP3), 160° (MP4SD), and 155° (MP4SDQ). Additional calculations with a larger basis set (MP2/6-31G*//HF/6-31G*) result in an energy minimum at C—C≡S = 148° and in a barrier to linearity of 1.5 kJ/mol corresponding to a thermal average of ⟨C—C≡S⟩ = 153°. This theoretical prediction is in good agreement with the electron diffraction experiment, which resulted in ⟨C—C≡S⟩ = 155 (3)° and in an estimated barrier of ≥2.0 kJ/mol.

Stereoselective Syntheses of the Nonactate Esters via Intramolecular Oxymercurations of Allenes [*J. Am. Chem. Soc.* **1990**, *112*, 1597]. ROBERT D. WALKUP* and GYOOSOON PARK

Our assertion that no previous syntheses of homononactic acid had been reported was incorrect, as we overlooked the first total syntheses of (+)- and (−)-methyl homononactate, as well as their utilization in the first total synthesis of tetranactin, by Schmidt and Werner: Schmidt, U.; Werner, J. *J. Chem. Soc., Chem. Commun.* **1986**, 996–998. Schmidt, U.; Werner, J. *Synthesis* **1986**, 986–992.

In addition, our discussion of the trends observed for the NMR spectra of the syn and anti diastereomers of our 1,3-diol intermediates **18–21** should have referenced the contributions of Hoffmann and Weidmann to this area of stereochemical analysis: Hoffmann, R. W.; Weidmann, U. *Chem. Ber.* **1985**, *118*, 3980–3992.