reduction in the 532-nm pigment band intensity (photolysis of 3-(diazoacetoxy)retinal in *n*-hexane under this condition led to total disappearance of its 245-nm band).

The extent of cross-linking, i.e., ca. 25%, was estimated by taking aliquots at suitable intervals during irradiation, denaturing the pigment by heating in SDS for 2-3 min, adding EtOH, and scintillation counting the pellet obtained by centrifugation.[30]

An advantage of the diazoacetoxy photoaffinity group is that its characteristic IR frequency around 2150 cm^{-1} is in a region normally transparent in biopolymers. Thus although the diazo band is too weak to be observed in the FTIR of the pigment prior to cross-linking (Figure 1, arrow), the difference spectrum measured after irradiation at 254 nm clearly shows the 2110-cm^{-1} band due to disappearance of the photoaffinity group (Figure 1, insert).[31] Studies are in progress to locate the site(s) of labeling in bR.[32]

Acknowledgment. We are grateful to J. D. Carriker for incubations and spectroscopic measurements, to A. A. Croteau for FTIR measurements, and to the NSF (Grant CHE 81-10505) for financial support.

Registry No. 1, 78324-68-2; [^{14}C]-**1**, 86309-92-4; 3-hydroxy-*trans*-retinal, 6890-91-1; [1-^{14}C]glyoxylic acid tosylhydrazone, 86309-93-5.

(30) We thank Drs. H. Bayley and K.-S. Huang for this procedure (to be published).

(31) The difference in frequencies of the diazo group in the unbound chromophore (2140 cm^{-1}) and bound chromophore (2110 cm^{-1}) is presumably due to environmental effects.

(32) Collaboration with Prof. H. G. Khorana and co-workers.

Evidence for the Necessity of Double Bond (13-Ene) Isomerization in the Proton Pumping of Bacteriorhodopsin

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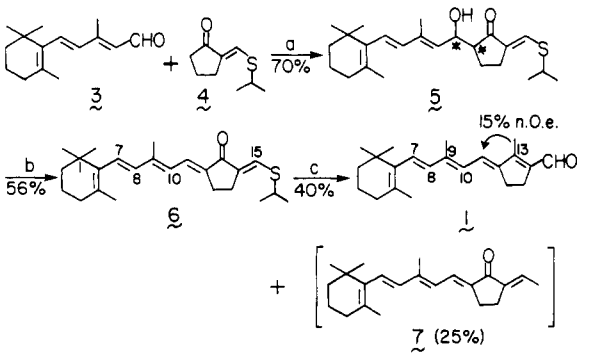
Bacteriorhodopsin (bR), the pigment of purple membrane (PM), converts solar energy into a proton gradient that is coupled to ATP synthesis.[1] bR consists of a protein (opsin) that binds one retinal molecule at Lys-216[2] through a protonated Schiff base linkage.[3,4] There are two modifications for bR,[5] the light- and dark-adapted forms, bR^{LA} (570 nm) and bR^{DA} (560 nm), the chromophores of which are *trans*-retinal and 1:1 mixture of *trans*- and 13-*cis*-retinals.[6] Although both forms undergo a photocycle, only that of bR^{LA} is associated with H^+ pumping.

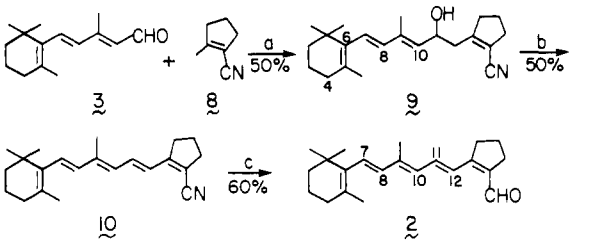
Proton translocation during the photocycle is thought to be associated with changes in the protonation state of the Schiff base linkage as well as retinal geometry. However, the structures of photocycle intermediates, e.g., M_{412} species, and their relation to the mechanism of proton pumping is not clear. Although resonance Raman and FTIR spectroscopy[3b,8a,b,d] have shown that the M_{412} species is not protonated, results pertaining to the nature of 13-ene in M_{412} are conflicting, i.e., it is a 1:1 mixture of cis/trans,[6a] 13-trans,[8b] or mostly 13-cis.[3b,7,8a,c] Therefore information pertinent to the molecular events involved in the proton pumping was sought by the study of retinals **1** and **2** with fixed 13-trans and 13-cis structures. The bR analogues derived from these retinals both *failed to pump protons, thus showing that the 13-ene isomerization appears to be necessary for proton translocation.*

Scheme I



[a] (i) **4** in LDA/THF -78 °C, 15 min; (ii) **3**, -78 °C, 20 min; (iii) 3 equiv of AcOH, -78 °C. [b] (i) $MsCl/Et_3N/CH_2Cl_2$, 0 °C, 1 h; (ii) flash chromatography. [c] (i) $MeLi/Et_2O$, -78 °C; (ii) satd aq NH_4Cl, -30 °C → 25 °C (30 min); (iii) flash chromatography.

Scheme II



[a] (i) LDA/THF, -78 °C → 25 °C (40 min); (ii) 2 equiv of HMPA, 0 °C; (iii) addition of **3**, -78 °C (1 h) → 0 °C (40 min). [b] (i) Ac_2O/py, 25 °C, 2 h; (ii) *t*-BuOK/THF, 0 °C, 30 min. [c] (i) DIBAL/Et_2O, -78 °C (1 h) → -40 °C; (ii) EtOAc, -40 °C, followed by aq $(COOH)_2$, -40 °C (15 min) → 25 °C.

The trans-fixed aldehyde **1** was synthesized according to Scheme I. The C_{15}-aldehyde **3** was condensed in aprotic medium with thiovinyl ketone **4** (from 2-(hydroxymethylidene)cyclopentanone[9] and 2-propanethiol,[10] mild conditions[11]) to give β-hydroxy ketone **5** as a 55:45 diastereomeric mixture (^{1}H NMR).[12] Dehydration of **5** with $MsCl/NEt_3$[13] provided thiovinyl ketone **6** as the major product: mp 130.5-132.0 °C (hexane); UV (hexane) 386 nm.[14]

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(9) Thompson, W. C. *J. Am. Chem. Soc.* **1931**, *53*, 3160-3164.

(10) The yield of **3** was greatly decreased by usage of 1-propanethiol or azeotropic removal of water.

(11) Bernstein, P. R. Ph.D. Thesis, Columbia Unversity, New York, NY, **1978**.

(12) All new compounds were characterized by ^{1}H NMR, UV, IR, and MS.

(13) Stork, G.; Kraus, G. A. *J. Am. Chem. Soc.* **1976**, *98*, 2351-2352.

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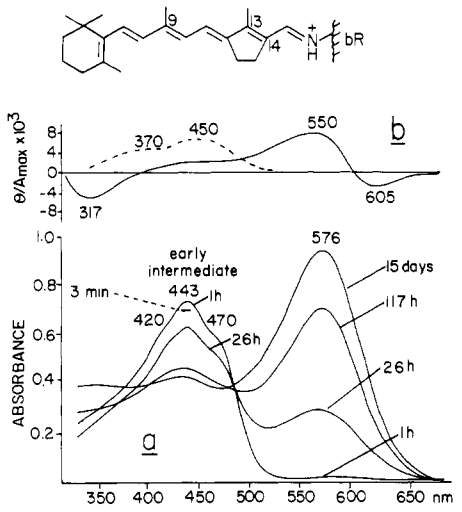

Figure 1. (a) Formation of bR-**1** from a 1:1 OD ratio of apomembrane and retinal **1**, in 10 nM Hepes buffer pH 7.0, dark, 22 °C. (b) Circular dichroism spectrum of bR-**1** after 15 days of regeneration; dotted line shows the spectrum of the early intermediate.

The 13-Me group was introduced by treatment with MeLi; the product, without isolation, was treated with aqueous NH_4Cl, which induced anionotropic rearrangement of the OH and elimination of mercaptan[15] to give, after flash chromatography, 40% **1** and 25% **7** (1,4-adduct). The trans structure of **1** is based on ^1H NMR data,[16] comparison of δ values with those of other double bond isomers,[17] and NOE results (see **1**). For the synthesis of cis-locked **2** (Scheme II), C_{15}-aldehyde **3** was condensed with 1-cyano-2-methylcyclopentene (**8**)[18] to afford nitrile **9**. Dehydration of **9** with Ac_2O/py[19] gave **10** as the only double bond isomer,[20] which was reduced and hydrolyzed[21] to desired aldehyde **2**: UV (hexane) 366 nm.[22]

The binding of *trans*-retinal **1** to the apoprotein[23] yielded within 3 min an "early intermediate" with fine structures at 420/443/470 nm (Figure 1) and CD maxima at 370 and 450 nm. The 443-nm UV peak is then slowly[24] replaced by a 576-nm bR^{DA} species, which peaks after 15 days (!). The similarity of the opsin shift (OS = 4140 cm^{-1}) to that of PM (4870 cm^{-1}),[26] reextraction of

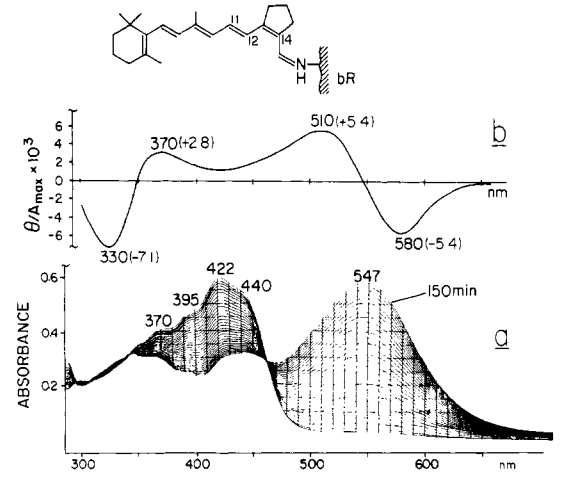

Figure 2. (a) Formation of bR-**2** from a 1:1 OD ratio of apomembrane and retinal **2**, in 10 mM Hepes buffer pH 7.0, dark, 22 °C. The maximum pigment yield is achieved after 66 h. (b) Circular dichroism spectrum of bR-**2** after 66-h incubation.

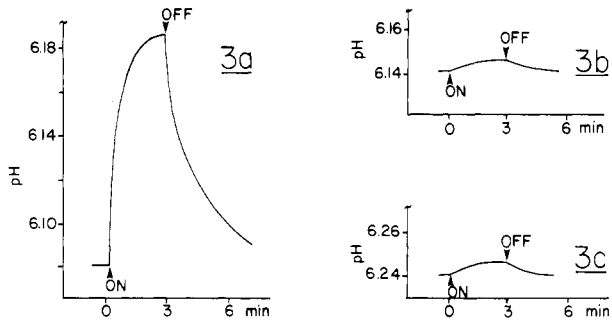

Figure 3. Proton translocation by (a) purple membrane; (b) BSA-washed apomembrane; (c) bR-**1** prepared from BSA-washed apomembrane. The pH of the medium (unbuffered 0.5 M KCl) containing the vesicles[29] (lipid/protein w/w, 60/1) was monitored with a glass electrode. Irradiation at >530 nm, 30 ± 0.1 °C; arrows show start and stop of irradiation.

authentic **1** by the CH_2Cl_2 procedure,[27] and the displacement of **1** by *trans*-retinal from the binding site indicate that the trans-fixed analogue **1** occupies the same binding site as natural *trans*-retinal.

The binding of retinal **2**, SBH^+ λ_{max} (MeOH) 440 nm, with the fixed 13-cis bond proceeded faster than for **1** (Figure 2). As in **1**, an intermediate was formed prior to the final 547-nm pigment, CD 580 nm (−5.4)/510 nm (+5.4). The OS value of 4480 cm^{-1} for bR^{DA}_2 is identical with that for $bR^{DA}_{13\text{-cis}}$.[26] A major difference between bR-**1** and bR-**2** is the photosensitivity of the latter; thus, irradiation with light of >530 nm, room temperature, caused 90% bleaching in 30 min.[28]

Vesicles prepared from bR-**1** and soybean phospholipids[29] were measured for their proton-pumping ability according to published procedures.[30] The amount of H^+ translocation resulting from irradiation of bR-**1** was negligible and is similar to that of BSA-washed apomembrane,[31] the blank (Figure 3); in both cases, the slight pH rise is attributed to a small amount of residual bR^{LA}.

(14) ^1H NMR of **6** ($CDCl_3$, 250 MHz) δ 7.59 (t, J = 3 Hz, 15-H), 7.46 (dt, J = 13, 3 Hz, 11-H), 6.41 (d, J = 16 Hz, 7-H), 6.23 (d, J = 16 Hz, 8-H), 6.18 (d, J = 13 Hz, 10-H), 3.33 (septet, J = 7 Hz, RSC*H*Me_2), 2.75 and 2.53 (4 H, cyclopentane), 2.08 (s, 9-Me), 1.70 (s, 5-Me), 1.41 (6 H, d, J = 7 Hz, *sec*-Me's), 1.04 (6 H, s, 1-Me).

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(16) ^1H NMR of **1** ($CDCl_3$, 250 MHz) δ 10.13 (s, CHO), 6.79 (br d, J = 12 Hz, 11-H), 6.31 (br d, J = 16 Hz, 7-H), 6.21 (d, J = 16 Hz, 8-H), 6.20 (d, J = 12 Hz, 10-H), 2.70 (br, 4 H, cyclopentane), 2.23 (s, 13-Me), 2.03 (s, 9-Me), 1.75 (s, 5-Me), 2.23–1.46 (br, 6 H, ionone), 1.05 (s, 1,1′-Me).

(17) Isomers of **1** with 9-cis and 11-cis double bonds have been synthesized and bound to bovine opsin: J.-M. Fang et al., manuscript in preparation.

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(20) Dehydration with $MsCl/Et_3N$ gave the nonconjugated 4,6,8,10-tetraene and **10** in a ratio of 5:1.

(21) Haeck, H. H.; Kralt, T. *Recl. Trav. Chim. Pays-Bas* **1966**, *85*, 343–346.

(22) ^1H NMR of **2** ($CDCl_3$, 250 MHz) δ 10.22 (s, CHO), 7.13 (d, J = 15 Hz, 12-H), 6.90 (dd, J = 15, 11 Hz, 11-H), 6.33 (d, J = 16 Hz, 7-H), 6.22 (d, J = 11 Hz, 10-H), 6.17 (d, J = 16 Hz, 8-H), 2.88–2.66 (4 H, cyclopentane), 2.02 (s, 9-Me), 1.73 (s, 5-Me), 1.04 (s, 1,1′-Me).

(23) Preparation of apoprotein and binding was performed similarly to the one previously reported, e.g.: Motto, M.; Sheves, M.; Tsujimoto, K.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1980**, *102*, 7947–7949.

(24) The long-living 443-nm intermediate is in contrast to that encountered in bR reconstitution (430 nm) which converts to bR in ca. 30 min at 0 °C: Schreckenbach, T.; Walckhoff, B.; Oesterhelt, D. *Eur. J. Biochem.* **1977**, *76*, 499–511. The nature of the 443-nm species and similar intermediates derived from 14-methyl analogues are under further study to clarify the process of bR formation.

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(26) Nakanishi, K.; Balogh-Nair, V.; Arnaboldi, M.; Tsujimoto, K.; Honig, B. *J. Am. Chem. Soc.* **1980**, *102*, 7945–7947.

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(28) It is interesting to note that *trans*- and 13-*cis*-14-methylretinal [Chan, W. K.; Nakanishi, K.; Ebrey, T. G.; Honig, B. *J. Am. Chem. Soc.* **1974**, *96*, 3642–3644], which are structurally related to **1** and **2**, did not give bR analogues upon incubation. However, both did immediately form intermediates with fine-structured bands at 410/428/450 and 370/390/410 nm, respectively. The 14-methylretinals are probably sterically more demanding than **1** and **2**.

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Results from bR-2 were similar except that due to its photosensitivity, alkalinization of the medium was monitored against irradiation time; the extent of H^+ pumping remained constant at the level of blank and thus it is also due to residual bR^{LA}.

The results described show that fixed 13-ene structures inhibit proton translocation. It has been shown that bR^{LA} formed from 5,6-dihydro-,[32] phenyl-,[33] and 3-(diazoacetoxy)retinal[34] still retain the ability to pump protons although less efficiently. This suggests that the 13-ene plays a more important role than the ring site in initiating the translocation of protons across the membrane.

Acknowledgment. The studies were supported by NSF Grant CHE 81-10505.

Registry No. 1, 86309-94-6; **2**, 86309-95-7; **3**, 3917-41-7; **4**, 86309-96-8; **5** (isomer 1), 86309-97-9; **5** (isomer 2), 86310-00-1; **6**, 86323-11-7; **7**, 86323-12-8; **8**, 765-76-4; **9**, 86309-98-0; **10**, 86309-99-1; hydrogen ion, 12408-02-5.

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Structure and Synthesis of 3-Deoxy-D-*glycero*-pentos-2-ulose, an Unusual Sugar Produced Enzymatically from (ADP-ribosyl)histone H2B

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Poly(ADP-ribosylation) is a posttranslational covalent modification of histones and non-histone nuclear proteins including poly(ADP-ribose) synthetase itself in eukaryotic cells.[1] It is initiated by enzymatic reactions of NAD on reactive functional groups of proteins such as glutamate of histones[2,3] followed by elongation and branching. Evidence suggests the involvement of poly(ADP-ribosylation) in various biological functions.[4-6] Although poly(ADP-ribose) is known to have α-ribosyl linkages at its C-2′ elongation sites[7] and C-2″ branching sites,[8] the nature of the histone/poly(ADP-ribose) linkage is not fully understood.[2,3]

We have purified and characterized ADP-ribosyl protein lyase, an enzyme that cleaves the ADP-ribose/histone linkage to give, instead of the expected ADP-ribose, an unidentified ADP-X.[9,10]

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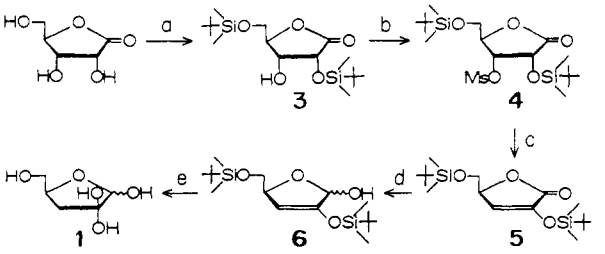
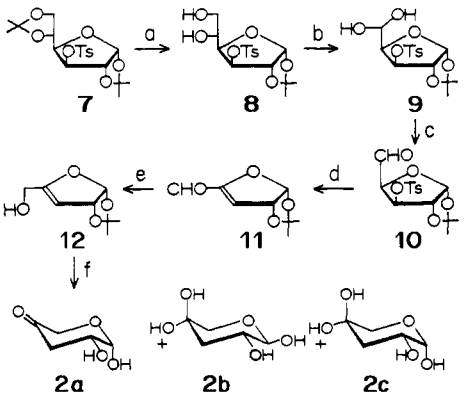
Scheme I



[a] *t*-BuMe$_2$SiCl/Py; Me$_2$NPy, room temperature, 4 h. [b] MsCl/Py; Me$_2$NPy, room temperature, 2 h. [c] Et$_3$N/C$_6$H$_6$, reflux, 2 h, 80% over 3 steps. [d] DIBAL/CH$_2$Cl$_2$, −78 °C, Ar, 64%. [e] Bu$_4$NF/THF, room temperature, 30 min, 38%.

Scheme II



[a] TsOH/MeOH, reflux, 4 h. [b] NaIO$_4$/MeOH-H$_2$O, room temperature. [c] C$_6$H$_6$, reflux, 1 h. [d] Et$_3$N/C$_6$H$_6$, reflux, 1 h, Ar, 67% from 7. [e] DIBAL/CH$_2$Cl$_2$, −78 °C, Ar, 80%. [f] AcOH-H$_2$O (2:1), room temperature, overnight, 76%.

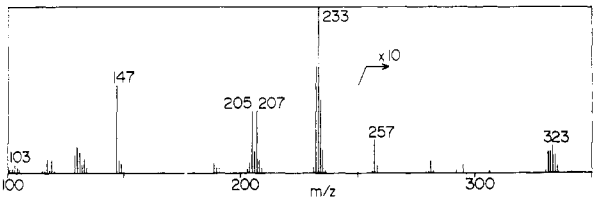


Figure 1. EI mass spectrum of a reduced X-d_2 Me$_4$Si derivative (erythro derivative; threo derivative showed almost identical spectrum).

In contrast, nonenzymatic cleavage of (ADP-ribosyl)histones yielded ADP-ribose.[2,3,11,12] The sugar X obtained by successive degradation of ADP-X with phosphodiesterase and phosphatase retains the five carbons of the ribosyl nicotanamide portion of NAD as shown by ^{14}C-labeling studies[10] but differs from the common pentoses.[10] Sugar X (ca. 10 μg using ca. 100 rat livers)[10] was reduced by NaBH$_4$[13] to the pentitol (reduced X) whose R_f value on paper chromatogram (R_f 0.51; *n*-BuOH/AcOH/H$_2$O 52:13:35 v/v[14])[10] suggested it to be 3-deoxypentitol.

Two of the most plausible candidates for X,[15] 3-deoxy-D-*glycero*-pentos-2-ulose (**1**)[16,17] and -4-ulose (**2**)[16,18] were therefore

(10) Nature of the substrate, biological details, etc. are discussed in the following: Oka, J.; Ueda, K.; Hayaishi, O.; Komura, H.; Nakanshi, K. *J. Biol. Chem.*, submitted for publication.
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(14) This solvent system generally does not distinguish epimeric alditols such as ribitol, arabinitol, and xylitol.
(15) The possibility of X being a 5-ulose was considered unlikely because 5-O is phosphorylated in the original (ADP-ribosyl)histone.

0002-7863/83/1505-5164$01.50/0